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A STUDY OF THE EPIDERMAL AND HYPODERMAL LAYERS OF THE ONION SEEDLING ROOT

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "A STUDY OF THE EPIDERMAL AND HYPODERMAL LAYERS OF THE ONION SEEDLING ROOTS", submitted by Pearl Gladys Ruth Kantor in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

Examination of the epidermis of the roots of onion seedlings revealed that the epidermis is undifferentiated and hairless in neutral tap water. The nature of the cell walls of these epidermal cells was studied microchemically.

Microchemical tests were masked by a xylene soluble compound. Studying the fatty compounds revealed substantial amounts of fatty substances in the meristematic cells although there is none in their cell walls. When mature, the entire epidermis including the root hair is covered with a cuticle. In general, the longer the epidermal cell the thinner the cuticle. This suggests that cutinization of the epidermal cell wall has an inhibiting effect on cell elongation.

The suberinization of the hypodermis begins on the outer tangential wall and proceeds to the radial walls, and then inwards to the inner tangential walls. Differences were noted in the composition of the long and short hypodermal cell walls, the short cells having less suberin and more pectin. This suberized, differentiated hypodermis never produces hairs even when under certain circumstances it forms the outermost layer.

Roots were grown in different solutions to determine the effects of various substances on root hair production. Calcium, in amounts greater than that found in tap water, represses root hair development. Acid conditions, on the

other hand, stimulate root hair formation and inhibit cutinization of the epidermis and suberization of the hypodermis. The chelating agents, Na_2EDTA and ammonium oxalate, stimulate swelling of the epidermal cells and the production of a variety of papillae and root hairs. Alkaline solutions of Na_2EDTA result in rupturing of the epidermal cells which can not be attributed to the formation of calcium pectate but to the development of a very thick cuticle. Lipoxygenase at pH 7 stimulates root hair development as does a deficiency of dissolved oxygen in the culture medium.

It was found that the formation of root hairs is not only dependent upon conditions that affect the pectic compounds but upon those that affect the fatty substances as well. The epidermal cell walls of the onion seedling root, strengthened by means of both the cuticle and calcium pectate, seem to be too stiff normally for root hairs to develop.

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INTRODUCTION

Onion roots have attracted attention by virtue of the fact that they are hairless in water (Farr, 1925) and produce root hairs in moist air only when they come in contact with moist filter paper (Rosene, 1954). Moreover, Mer (1884) states that they produce hairs rarely over long periods of time in moist air.

It should be noted here that the common onion produces two sets of roots, namely the seedling or primary root, and the bulb or adventitious roots. Failure to recognize this condition has led to considerable confusion in the past. Observations reported in the present investigation have been obtained from the study of seedling roots exclusively.

The purpose of this investigation is to find out if onion seedling roots do form root hairs, and if they do, to determine the conditions necessary for their development. For convenience the subject matter is divided into four parts:

A. The culture and growth of onion seedling roots. (standard procedure)

B. The anatomy of the seedling root.

C. Microchemical studies.

D. Effects of various culture solutions on root growth and root hair development.

LITERATURE REVIEW

Because of their size and the ease with which they can be grown, root tips of onion are commonly used to study the principal stages of tissue differentiation: cell division, cell elongation, and cell maturation. Since the literature in this regard is voluminous and very diverse, references will be made only to those papers which have a direct bearing on the problem at hand. Relatively little work has been done on the epidermis and hypodermis, *per se*, and what has been done has been carried out with bulb roots (Esau, 1953; Mer, 1884; Rosene, 1954; Scott *et al.*, 1958).

However, Hoffman (1933) and Hayward (1938) found that the development of the root axis of both seedling and bulb roots is exactly the same, and it has been assumed that cellular morphogenesis is also the same in both kinds of roots.

A. The Culture and Growth of Onion Seedling Roots.

Sideris (1925) was the first to take notice of the fact that the common onion produces two sets of roots. One, the seedling or primary root originates on germination and dies at about the time of the formation of the bulb. He believed that death of the seedling root is due to three causes: senility of the stem tissues, senility of the root, convolutions in the stem due to irregular growth between

old and new tissues resulting in the disconnection of both water and food supply at the time of bulb formation. The second set of roots are the so-called bulb roots. These are adventitious roots and arise in succession from the center of the stem to the periphery.

Developmental morphology of both seedling and bulb roots was examined by Hoffman (1933). His extensive study described the germination of the seed, the formation of the bend in the cotyledon called the "knee", and the development and morphology of the root systems, as well as that of the leaves and bulb.

He described the meristem of the seedling root as having two regions, one producing the stele, and one producing the cortex, the epidermis, and the root cap. In the latter meristematic region, the lowermost layer of cells gives rise to the root cap, while a somewhat higher layer gives rise to the epidermis. In this same meristematic region another layer of cells, by several periclinal divisions, results in the development of a cortex some five to eight cells in width. Numerous transverse divisions give rise to cortical cells shorter than those of the stele and longer than those of the epidermis. Both the cortex and the epidermis are parenchymatous.

Hoffman also examined the development of the adventitious roots which first originate in the pericyclic region of the stem at the level of the apical meristem. He found that the development of the axis of a bulb root is exactly

the same as the axis of the primary root, except that each region of the bulb root is larger because of a correspondingly greater number of cells in the two meristematic regions. The primary root differs from the adventitious root in several features. Whereas the stele of the seedling root is usually diarch or triarch, the bulb root is tetrarch, pentarch, or hexarch. Also, the seedling root never forms a secondary root although the bulb root occasionally does.

Hoffman's (1933) and Hayward's (1938) studies on germination and seedling growth showed that after about twenty-four hours, due to the elongation of the lower and middle parts of the cotyledon, the hypocotyl emerges through the micropyle. This in turn is followed by the cotyledon with the exception of its tip. This portion of the cotyledon remains embedded in the endosperm and thus acts as a haustorium for the absorption of food. At first the emerging root tip grows upward but when the embryo outside of the seed reaches a length of from four to six millimeters, a bending occurs in the cotyledon which directs the cotyledon downwards. This results in the formation of a sharp bend in the cotyledon which is called the "knee". One portion of this "knee" leads to the terminal portion of the cotyledon, embedded in the endosperm, and the other portion leads to the cotyledonary plate. Subsequent elongation of both portions of the cotyledon brings the "knee" above the surface of the soil. Once above the surface of the soil the portion of the knee next to the seed ceases to elongate

while the portion of the knee next to the stem region continues to elongate. The unequal growth of these two portions of the knee causes the cotyledon to assume the shape of a bow, with the stem limb curved and the seed limb stretched across this curved portion like a bowstring. The resulting tension draws the tip of the cotyledon out of the seed and above the surface of the soil. As the cotyledon straightens out, a slight kink remains at the site of the "knee".

B. The Anatomy of the Seedling Root

Very little has been published on the tendency of onion root cells to produce root hairs. Mer (1884) observed that onion bulb roots are hairless in water, and produce hairs rarely in moist air or sand. He also noted that the occurrence of these hairs is highly irregular. Rosene (1954) found that onion bulb roots do not tend to form hairs unless the roots are in contact with moist filter paper. Bulb root epidermal cells were studied by Scott *et al.* (1958). They found no root hairs although occasionally papillae were evident in the region of differentiation. Guttenberg (1943) described the long and short hypodermal cells. According to his report the long cells are suberized but the short cells are not.

In their studies carried out on onion bulb root tip cells, Jensen and Kavaljian (1958) showed that immediately above the apical meristem the cortical and protoderm cells

undergo an extensive radial enlargement. While at this stage the pro-vascular cells increase only slightly in diameter. After radial enlargement has virtually ceased cell elongation begins in the pro-vascular, cortical, and protodermal tissue. As a rule cell volume increases moderately at first due to the increase in cross sectional area, and then much more rapidly due to increased cell elongation. They also found that in the root cap cells apical to the meristem, increase in cell volume is due to radial enlargement; and in those cells basal to the meristem it is due to cell elongation. Cell volume is not only a function of cell growth but also of cell division. By counting dividing nuclei, they revealed that there are differences in the various tissues, in distances from the tip at which the greatest frequency of cell division occur. In agreement with Clowes (1956a, 1956b) who clearly demonstrated a region of very low DNA formation, and Peterson (1965), they found the existence of a quiescent center in which few, if any, cell divisions occur and which is surrounded by tissue in which some divisions are occurring. Further back from the root tip, behind the quiescent center, the cortex is actively dividing. Even further back there is a zone in the stele with many divisions. Finally the epidermal cells which have been dividing all along enter their period of highest cell division.

Attempting to correlate these results with nucleic acids and protein content, Jensen (1958b) found in onion

bulb roots that in the region in which radial enlargement is very rapid DNA increases to a constant value about twice that found in the quiescent center. RNA increase is directly proportional to cell size in this area of radial enlargement, and protein is also increasing sharply. After the increase in all three fractions there is a leveling off in the content of DNA, RNA, and protein in the zone of elongation, followed by an increase of protein during the final period of rapid elongation (Jensen, 1958b).

Scott *et al.* (1956) made an intensive study of cell shape and cell wall structure of onion bulb root tips. A special feature of this study undertaken by means of both the light and electron microscope was the discovery of minute circular pits in the walls of the apical initials. The cells are polyhedral in shape and about 10 μ in diameter, and the numerous pores, about 1 μ in diameter, give to every cell face a sieve-like appearance. Intercellular spaces make their first appearance about 20 μ above the apical initials. By this time the cells are drum-shaped and each cell is rounded at the corners and on all vertical surfaces adjacent to the intercellular spaces. On the pit areas, however, the walls are closely adpressed and thus appear flattened. The end walls are also finely pitted, the pits being roughly radially distributed. On the vertical walls the closely set elliptical pits are diffuse at first, but soon become lined up in the areas of contact with the walls of the adjacent cells. In turn the many ranked

elliptical pits become linear making the vertical wall appear scalariform pitted. As the intercellular spaces enlarge, non-pitted areas appear between the pitted bands, and the pits themselves become larger and reorientated in single file. The pits increase in size during this re-orientation to an ellipical area 2 μ by 5 μ which may be randomly subdivided by cellulose strands of varying thickness. When the region of elongation is reached the vertical walls increase greatly in length and the pits are still in vertical alignment and generally in single file or in small groups, although the end walls are still sieve like. In this region the plasmodesmata and the ubiquitous suberin pellicle, described earlier by Scott and Lewis (1953), are evident.

In the region of differentiation, and in the root hair zone Scott *et al.* (1956) found the pattern of pit distribution unchanged. They saw that secondary walls are laid down after the wall has ceased to enlarge which supports the views of Esau (1953). The end walls also undergo an appreciable degree of secondary thickening.

Scott *et al.* (1956) also correlated their electron microscopic examinations with light microscopic studies, and described pit development in detail. They concluded that the microfibrillar pattern of the primary wall passes through three phases: first, the interwoven mesh of the apical initials; second, the parallel orientation of the main part of the meristem and the zone of active elongation; third, the final reticulum of the post-elongation, pre-

secondary thickening cells. The deposition of the secondary thickening is layer by layer. The first layer consists of closely set microfibrillae, the first of which may be somewhat distant from each other and the gaps may be closed by later deposition. Successive sheets of microfibrillae, helical alternately clockwise and counterclockwise, produce a crisscross design. This agrees with the pattern described by Frey-Wyssling (1953). There is no evidence of active bipolar growth during cell elongation, in opposition to the concept of bipolar growth advanced by Mühlethaler (1950) to account for cell elongation.

The fine structure of onion bulb root tip cells was also studied by Branton and Moor (1964), but their work revealed nothing of the wall structure. They did report that in root cap cells small Golgi vesicles accumulate on the outside of the plasmalemma, in agreement with a previous observation in maize root tips (Mollenhauer *et al.*, 1961).

C. Microchemical Studies.

Using onion bulb roots for the analysis of carbohydrate and hexose, Jensen (1958a) indicated that in the root cap there is a high carbohydrate and hexose content due to heavy cell walls of the root cap cells. In the apical meristem region there is a decrease in carbohydrate content of the cells. As the volume of the cell increases so does the carbohydrate content indicating that the total carbohydrate present is directly proportional to the cell volume. In

the zone of rapid cell elongation, the amount of carbohydrates per cell increases more rapidly than the total amount of hexose. This would suggest that after cell elongation begins, there is an increase in the formation of non-hexose containing cell-wall components (Jensen, 1958a). This is in agreement with the results obtained in studies of the roots of *Vicia faba* (Jensen, 1955) and corn (Dever *et al.*, 1968).

Histochemical analysis of the onion bulb root cell wall (Jensen, 1960, and Jensen and Ashton, 1960) showed that the rootcap cell wall is the most massive and is high in cellulose and soluble non-cellulosic polysaccharides, high in water soluble hexoses, pentoses, and hexuronic acid and pectin; and low in proto-pectin, hemicellulose, and insoluble non-cellulosic polysaccharides. The apical initials are low in all cell wall components, while cellulose and pectic components are present in equal amounts. When radial enlargement begins, the walls are low in all components, but the amounts of the major components are about equal overall, however, differences appear in the composition of the walls of various tissues. During radial enlargement differences in wall development between the protoderm and cortex appear. The protoderm has a slower rate of synthesis of non-cellulosic polysaccharides, and cellulose, and a higher rate of synthesis of pectic substances and hemicellulose. The differences between protoderm and cortex are lost during elongation, and finally the two tissues are similar except the outer wall of the protoderm

which is different than the other walls of the protoderm and resembles the walls of the rootcap in being very thick and high in cellulose.

Early in their development cortical cells have walls relatively rich in both noncellulosic polysaccharides and cellulose, and low in pectic substances and hemicellulose. During radial enlargement the rate of increase in wall materials is slow but, it is rapid during elongation. Wall thickening begins in the cortical cells nearest the center of the root and continues outwards from here. In general, the cortical cell wall is high in noncellulosic polysaccharides and cellulose, and low in pectic substances and hemicellulose.

Except for the metaxylem cells, the protovascular cells undergo little radial enlargement, and elongation begins much nearer the tip, at 400-500 μ, than it occurs in the cells of the cortex or protoderm. The walls of the provascular cells are low in noncellulosic polysaccharides, and cellulose, high in pectic substances initially, and hemicellulose later. Walls in which lignin will later form are those in which the noncellulosic polysaccharides and cellulose content is low. The cell walls of the protophloem elements have a very high cellulose content.

These complex changes in the chemical composition of the cell wall were correlated to changes in the morphology of the wall, (Jensen, 1958a, 1960; Jensen and Ashton, 1960). During radial enlargement the wall is morphologically a

loose open structure with large gaps, the primary pit fields, running at right angles to the long axis of the cell. During the stage in which radial enlargement is still occurring but elongation has begun the wall changes to a more continuous structure with the larger gaps being filled in, although still visible, and the primary pit field becoming smaller. This change is accompanied by an increase in all components per cell, but on a per unit area basis, only pectin showed an increase that could be correlated with elongation. When radial enlargement ceased and elongation was occurring there is a marked increase in the amount of cellulose, pectin, soluble hexose noncellulosic polysaccharide, and insoluble noncellulosic polysaccharide per unit area which can be correlated to morphological changes in the wall. The wall becomes continuous with all but the final traces of the primary pit fields invisible. That is, the gaps in the cell wall associated with radial enlargement are being filled in with cellulose, pectin, hemicellulose, soluble hexose noncellulosic polysaccharide, and the insoluble noncellulosic polysaccharide.

Scott *et al.* (1958) found pectin substances in the epidermal cell wall framework, particularly in the thicker outer wall.

With respect to the function of the various components of the cell wall Jensen (1960) suggests that since shrinkage in cell diameter occurs after removal of noncellulosic polysaccharides the cellulose microfibrils may actually be

under tension. He also suggests that cellulose is of critical importance in the structure of the wall, but that noncellulosic polysaccharides play a major role in determining the physical characteristics of the wall.

According to Scott *et al.* (1956) the suberin pellicle lining the intercellular spaces of the cortex of onion bulb roots resembles the cuticle of the bulb scale of onion in general appearance (Scott *et al.*, 1957, 1958). Presumably the suberin precursors in liquid state exude across unpitted cell wall areas adjacent to intercellular spaces and there harden on the wall surface (Priestly and Woffenden, 1922). Within the intercellular spaces Sorokin (1958) clearly showed the presence of an intercellular tubular material in addition to the usual intercellular lining. This intercellular tubular matter seems to be composed of an outer lipid membrane, a reducing sugar, an unidentified inner material, and air (Peterson, 1964; and Sorokin, 1958).

Scott *et al.* (1958) found that all the water grown onion bulb roots are coated with a mucilagenous material which is a colorless matrix with innumerable granules a fraction of a μ, along with sparsely scattered oil-like droplets. After several hours the matrix may stain faintly with ruthenium red, and most of the droplets redden with Sudan III.

Beneath the mucilagenous envelope lies the cuticle, which is very thin near the tip but is evident in the mature region and increases with age until at two to three

centimeters from the root tip the epidermal and sub-epidermal cell walls are completely cutinized (Scott *et al.*, 1958). This view disagrees with that of Guttenberg (1943), who states that only the short cells are suberized. Along the radial walls Scott *et al.* (1958) found fringed flanges of cutin. Scott (1950) and Scott *et al.* (1958) found in work on many different species of plants that all root hairs of all lengths have a cuticle.

In young cells, plasmodesmata radiate from the protoplast to all cell faces, including the outer where they appear to end in the cuticle. In older cells, pits on the outer and inner tangential walls are circular in outline, those on the cutin-fringed anticlinal walls are linear, and those on the end-walls are sieve-like.

With the electron microscope Scott *et al.* (1958) observed cuticular strands anchoring the cuticle to the cellulose framework of the wall. There is also a transition between the dominantly pectic and dominantly cellulose layer of the outer cell wall.

These same workers believed that the mucilagenous sheath could be the counterpart of the wax on the leaf surface and is, therefore, presumably extruded along the plasmodesmata through the cellulose-pectic wall and the cuticular layer (Scott *et al.*, 1958).

D. Effects of Various Culture Solutions on Root Growth and Root Hair Development.

A common method of studying the chemical make-up of the epidermal cell wall and its effect upon differentiation of the cell is to grow roots in various culture solutions which change the chemical composition of the cell walls. Manipulating the amount of calcium, the amount of pectin, and the pH results in drastic changes in the shape and form of the epidermal cells. These experiments and more recent experiments on the formation of callus on the stems of Balsam poplar cuttings (Cormack, 1965) indicate that calcium is necessary for the stiffening of the cell wall, and the pH regulates the rate at which calcification of the wall takes place. The effects of calcium and pH on roots and root hairs have been the subject of extensive research (Farr, 1928, 1929; Cormack, 1935-62; Bürstrom, 1952; Ekdahl, 1957a). Briefly the results may be summarized by stating that in general, acid conditions result in a very slow transformation of pectic acid to the rigid calcium pectate. The epidermal cells, hence, tend to be long and hairless, or at least sparsely haired. In culture solutions with calcium at neutrality or slightly alkaline, calcification of the cell walls occurs in some cells but not in others. As the calcium containing solution becomes more basic calcium pectate formation is rapid and generally epidermal cell elongation is inhibited and root hair development is stimulated. A further increase of pH, resulting in a strongly

basic calcium solution often results in the rupture of the epidermal cells due to their being very rigid from calcium pectate deposits.

Generally, the use of chelating agents in root culture solutions results in swollen epidermal cells in which the outer cell walls are variously pushed out. Ekdahl (1957a) did not observe this swelling of the epidermal cells in the roots of wheat. Two of the most common chelating agents used have been ammonium oxalate and disodium ethylenediamine-tetraacetate. Using ammonium oxalate, Cormack (1935) found that ammonium oxalate represses the formation of root hairs in *Brassica*. Ekdahl (1957a), growing wheat roots in solutions containing ammonium oxalate, noted that root hairs are formed but that they are shorter than those produced in solutions without oxalate.

The other common calcium chelating agent, EDTA, also represses root hair formation in *Brassica* (Cormack, 1959a). Varying degrees of abnormal swelling of the epidermal cells were also observed. EDTA also acts on the middle lamella to result in the maceration of plant cells (Letham, 1960).

These studies of the nature of the cell wall conducted by growing roots in various solutions with different amounts of free calcium and different pH's, have been supported by experiments with the enzyme pectinase (Cormack, 1955, 1956). Jackson (1959) felt that some of the results of these experiments using enzymes may have been due to impurities in the enzymes. Ekdahl (1957b) concluded from the results of

his studies using pectinase and cellulase that the "root hair wall is hardened by a formation of new cellulose strands in the primary walls of the growing hair apices."

Although roots have been grown in pectinase and cellulase to determine the effect of these enzymes on differentiation, no studies have been conducted on the effect of the enzyme lipoxidase on root hair development. That this enzyme plays a role in the oxidation of fatty acids to form suberin in the walls of the endodermis has been stated by Van Fleet in 1961. Accoding to Siddigi and Tappel (1956) lipoxidase may also play a role in the oxidation of fatty acids to form cutin. Fritz *et al.* (1958) have shown that lipoxidase catalyzes a reaction in which free oxygen is incorporated directly into organic material which is chloroform soluble.

According to Tappel (1962) the optimum pH of this enzyme is 6.5 to 7.0, although it also functions well at a pH of 9.0. The work of Van Fleet (1961) also supports the contention that suberization takes place most readily in a basic or neutral medium, and that the normal acid condition around the passage cells prevents suberin formation.

Cormack (1937) showed that *Elodea* roots developed in water and in light are green and possess a cuticle, while those growing in darkness, whether in water or in soil are colourless and have no cuticle. The oxygen set free by photosynthesis in light is thought necessary for the oxidation of fatty acids to form a cuticle. Once formed the cuticle prevents the epidermal cells from pushing out to

form hairs. In the absence of free oxygen the fatty acids are not oxidized, no cuticle results, and the elongating epidermal cells push out to form hairs. Dale (1951) studying the effects of the fatty acids and the cuticle on root hair development in *Elodea* roots also found that when the fatty acids are not oxidized on the epidermis to form a cuticle, root hairs result. The results of the earlier work on the effect of oxygen on root hair development are controversial and inconclusive.

MATERIALS AND METHODS

Plant Material

The variety Southport Red Globe of the cultivated onion (*Allium cepa* L.) was chosen as the experimental plant material. At first, experiments were carried out with both seedling and bulb roots in order to decide which kind of root would be most suitable. The seedling or primary root as the name suggests emerges at the time of germination and persists until the formation of the bulb, a period of two and one half months in water and about three months in soil. Bulb roots, on the other hand, develop in succession at the periphery of the bulb and function throughout the life of the plant.

Although most of the earlier work (Branton and Moor, 1964; Jensen, 1958a, 1958b, 1960; Jensen and Ashton, 1960; Jensen and Kavaljian, 1958; Mer, 1884; Rosene, 1954; Scott *et al.*, 1956, 1958) was carried out with bulb roots, such roots because of their manner of growth and lack of uniformity proved unsuitable for use in the present investigation.

Consequently, seedling roots were used exclusively as the experimental material.

Methods

A. The Culture and Growth of Onion Seedling Roots.

1. Seed germination and early seedling growth.

After washing with several changes of water, seeds

were soaked in warm water, treated with Ceresan M2x and placed on moist filter paper in petri dishes to germinate. Although this is the customary method of obtaining young seedling roots (Cormack, 1935-1962; Lemay, 1965; Peterson, 1964) all attempts to germinate and to grow onion seedlings on moist filter paper were unsuccessful. In the first place, onion seedlings, following germination, grow upwards, away from the moist surface of the filter paper, and as a result the delicate roots soon dry out. Secondly, on contact with moist filter paper, the fungicide, Ceresan M2x has an inhibiting effect on the germination and subsequent growth of the young onion seedlings. Consequently, in the present investigation, following washing and treatment with Ceresan M2x, seeds were sown in moist sand or vermiculite, at pH 7, as determined by a Truog soil reaction test.

2. Standard procedure

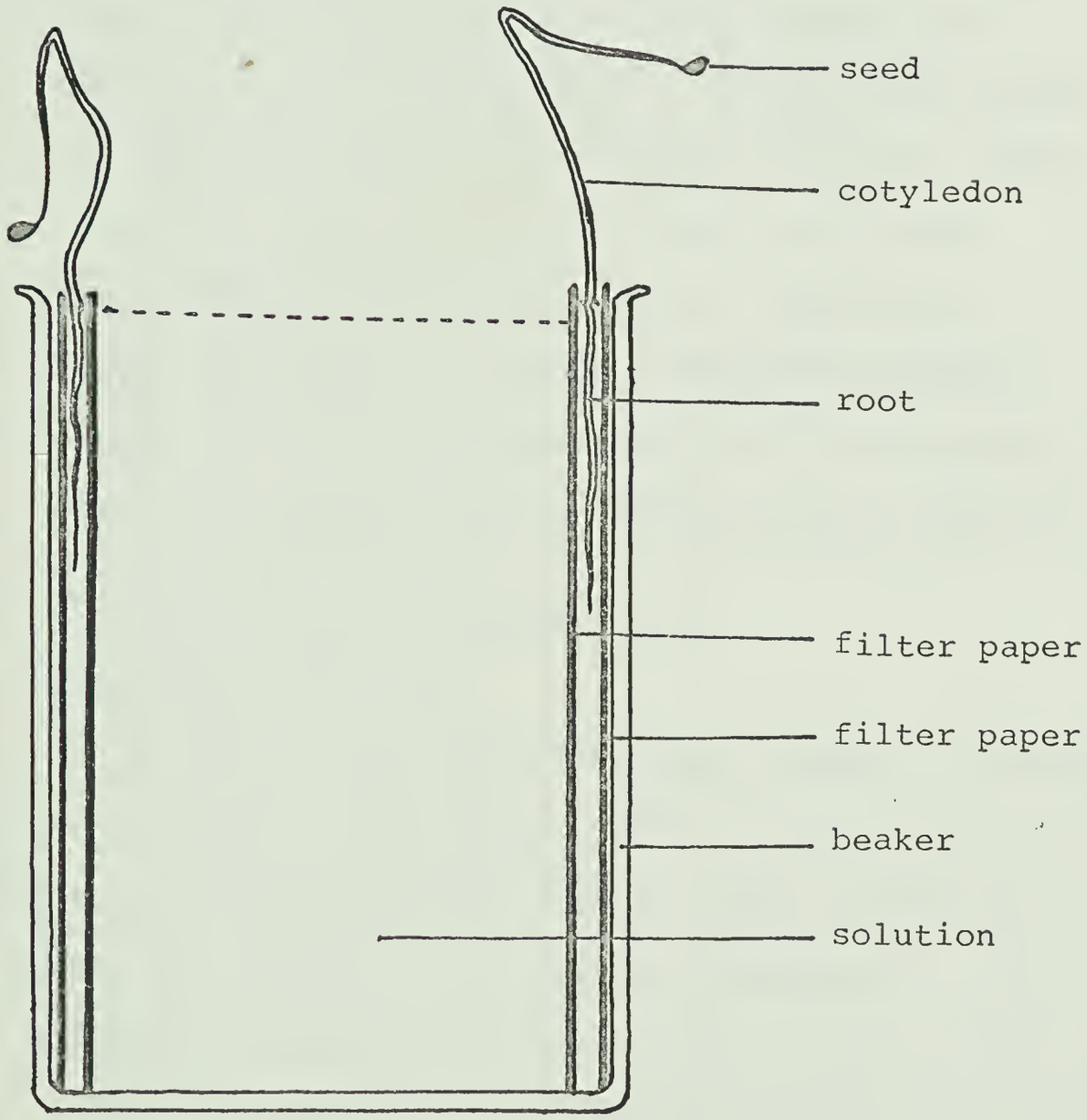
All attempts to produce root hairs on onion seedling roots in the customary manner (Cormack, 1935-1962), with their roots submerged in an aqueous solution were unsuccessful. After transfer to the aqueous solution, the seedling roots continued to grow but remained hairless.

Since Rosene (1954) showed that contact with moist filter paper was necessary for the production of root hairs on onion bulb roots, a method

FIGURE 1

Diagram showing the method of growing young seedling roots between two sheets of filter paper in a beaker containing the culture solution.

FIGURE I


seed
cotyledon
root
filter paper
filter paper
beaker
solution

was devised which enabled seedling roots to grow submerged in an aqueous solution and yet grow in contact with filter paper at the same time. The general procedure is as follows: seedlings that had developed to the stage at which the knee had begun to straighten out were removed from the sand, washed in water and placed between two sheets of moist filter paper, on which the length of the root was marked in pencil. The two sheets of filter paper were then placed in a beaker containing enough of the aqueous solution to cover the roots, the roots growing vertically downward between the two sheets of filter paper, into the aqueous solution for five days (Figure 1).

B. The Anatomy of the Seedling Root.

Preparation of slides.

In order to follow developmental changes in the epidermis of a multicellular organ, such as a root, it is desirable to try to examine this single layer of cells by itself. However, all attempts to do so by stripping off thin pieces of epidermis by means of a sharp razor blade, as Scott *et al.* (1958) discovered, or by clearing the root with various clearing reagents were unsuccessful. To overcome these difficulties, seedling roots were placed in 50% Chromic acid for a few minutes, and then washed thoroughly in several changes of distilled water. By this method the cellular material could be removed, leaving the epidermis,

hypodermis, and xylem intact and readily observable when mounted in glycerine jelly. These roots were compared with control roots which were sliced in half, longitudinally with a razor blade, stained briefly with methylene blue, and washed thoroughly with water. These controls make certain that the papillae and root hairs had not been removed by the drastic action of the chromic acid.

Examination of the prepared slides was facilitated by the use of a Reichert research microscope.

C. Microchemical Studies.

Roots for study were grown under the conditions previously described in section A, Standard Procedure, in tap water. In order to produce root hairs on which to perform the microchemical tests, some roots were treated as above with a modification such that, instead of being submerged in 80 milliliters of water, the roots were suspended between two sheets of moist filter paper which were kept moist by being in 10 milliliters of water (Figure 1).

For these histochemical tests fresh roots, roots which had been in xylene for a period of two weeks, and roots which had been in xylene for a much longer period of time were used. Since epidermal strips were unobtainable the entire root, longitudinal sections, and 20-28 μ thick cross sections were tested. Attempts were made to use the kryostat but mechanical difficulties made it impossible to obtain sections.

Two well known microchemical tests were used to test for the presence of cellulose: Zinc-chlor-iodine (Jensen, 1962), and the IKI-H_2SO_4 method in which either whole roots or sections were placed in IKI solution and then in 65% H_2SO_4 (Jensen, 1962).

To test for total lipids, roots were treated with Sudan IV in 70% ethanol and then washed with 50% ethanol, after the method described by Johansen (1940). Another test was performed in which the material was treated with 50% Chromic acid and then washed with water. This was used to determine the presence of cutin and suberin (Johansen, 1940).

Ruthenium red was used to test for pectic substances (Johansen, 1940). Attempts to discover the location of pectic acid were made by treatment in hydrochloric acid followed by staining with ruthenium red. Finally, after treatment with hydrochloric acid, the roots were placed in ammonium hydroxide and then ruthenium red. Although ruthenium red is not specific for pectic compounds it is a commonly used dye. The phloroglucin-HCl test for lignins was used also (Johanson, 1940).

D. Effects of Various Culture Solutions on Root Growth and on Root-Hair Development.

Besides microchemical testing for specific cell wall materials another method of probing into the chemical nature of the cell wall is to grow seedling roots in various culture solutions designed to change the normal chemical composition of the cell wall.

By means of this method (Cormack, 1935-1962) it had been shown in seedling roots that marked changes in cell shape, form, and arrangement can be brought about by manipulating the amount of calcium, and the pH of the culture solution, by the use of solutions of pectic enzymes, and by the use of calcium-chelating solutions. From the results of these experiments and from the results of more recent experiments with callus formation on the stems of Balsam poplar cuttings (Cormack *et al.*, 1965) it was concluded that calcium is necessary for the stiffening of the cell wall and that the pH regulates the rate at which calcification of the cell wall takes place. The present series of experiments were undertaken to discover to what extent the reactions of onion seedling roots would correspond to those of *Brassica* which were the main objects of the earlier work (Cormack, 1947, 1961; Cormack *et al.*, 1963; Peterson, 1964).

Seedlings which had grown to the stage where the knee was fully developed, furnished the experimental material for this study. When the seedling roots measured 7 to 12 millimeters in length they were treated in the same manner described already under the heading Standard Growth Procedure, the roots growing down into the culture solution between two pieces of filter paper (Figure 1). Growth in length of each seedling root was determined by marking with pencil the position of the root tip on the filter paper before and at the end of each experiment. Following growth in each culture solution, some of the roots were mounted

on slides and treated with chromic acid in the manner described already, while control roots were stained with methylene blue and mounted on slides in glycerine jelly.

1. Effect of Calcium and pH.

To determine the effect of availability of calcium and pH on root growth and root-hair development, seedling roots were grown in:

a. tap water

b. a saturated solution of calcium sulphate.

The pH was regulated from 4.5 to 9.0 by means of phosphate buffers.

2. Effect of Calcium Deficiency.

To test whether the failure of onion seedling roots to produce root-hairs in aqueous solutions is due to too much calcium in the solution resulting in the too rapid incorporation of calcium into the elongating cell walls, seedling roots were grown in the following calcium chelating solutions:

a. Disodium ethylenediaminetetracetate (Na_2EDTA).

b. Ammonium oxalate.

a. Disodium Ethylenediaminetetracetate (Na_2EDTA)

Five experiments were designed for the growth of seedling roots in solutions of Na_2EDTA. The following solutions were used:

(i) Na_2EDTA dissolved in distilled water at a concentration of $10^{-6 \cdot 5}M$ to $10^{-3 \cdot 5}M$.

(ii) Na_2EDTA dissolved in tap water at a concentration of $10^{-6 \cdot 5}M$ to $10^{-3 \cdot 5}M$.

(iii) Na_2EDTA dissolved in a saturated solution of calcium sulphate at a concentration of $10^{-6 \cdot 5}M$ to $10^{-3 \cdot 5}M$.

(iv) A solution of $10^{-4 \cdot 5}M$ Na_2EDTA in tap water with a pH ranging from 4.5 to 9.0, varied by means of phosphate buffers.

(v) A solution of $10^{-4 \cdot 5}M$ Na_2EDTA in a saturated calcium sulphate solution with a pH ranging from 4.5 to 9.0, varied by means of phosphate buffers.

b. Ammonium oxalate.

In this set of experiments, ammonium oxalate was added to the following solutions at a concentration ranging from $10^{-7 \cdot 5}M$ to $10^{-1 \cdot 5}M$.

(i) Ammonium oxalate in distilled water.

(ii) Ammonium oxalate in tap water.

(iii) Ammonium oxalate in a saturated solution of calcium sulphate.

3. Effect of Lipoxygenase derived from Soybean.

The presence of waxy substances in hypodermal, epidermal, and root-hair cell walls suggested that changes in cell shape, size, and arrangement might result by growing seedling roots in solutions of fat-splitting enzymes.

Three compounds were tried: Lipase (448), Lipase (Steapsin), and Lipoxygenase. The results of experiments with the first two enzymes were inconclusive while those with Lipoxygenase were most interesting. This enzyme, listed in the catalogue of Worthington Biochemical Corporation as the "fatty acid" or "linoleic acid" lipoxidase (Koch, Stern and Ferrari, 1958), is considered by Siddigi and Tappel (1956) to play a role in the formation of cutin. In an earlier catalogue the optimum pH for lipoxygenase was reported as 9.0, but in the 1968 catalogue this was changed to 6.5 to 7.0. After much experimentation two solutions of the enzyme were used: one at pH 6.0, and another at pH 7.0. The latter solution gave the best results. In this case the pH was adjusted by adding small amounts of sodium hydroxide to a solution of the enzyme in tap water. After much experimentation, tap water proved to be the most suitable solvent for this enzyme in concentrations of 0.5 and 1.0 mg of enzyme per liter of tap water. To assure that the enzyme was active the solution was changed daily.

4. Effect of Oxygen.

Since oxygen was found to play an important role in the formation of a cuticle on the epidermis of *Elodea* roots, (Cormack, 1937), experiments were conducted to test the effects of varying the concentration of oxygen in tap water. After considerable experimentation the following neutral aqueous solutions were used.

i. Boiled tap water

ii. Slightly aerated boiled tap water

iii. Aerated unboiled tap water

RESULTS

A. The Culture and Growth of Onion Seedling Roots.

1. Seed Germination and Early Seedling Growth

The results of preliminary experiments showed that washing the seeds prior to sowing on moist sand greatly speeds up the rate of germination. Dry seeds germinate in three to five days, while seeds soaked in several changes of warm water for two or three hours prior to sowing germinate in one or two days.

With minor exceptions, observations on the germination and early growth of the seedling confirm those of Hoffman (1933) and Hayward (1938) described already under the heading of Literature Review A. They also confirm the conclusion of Hayward (1938) that the development of the so-called "knee" results from the downward bending of the hypocotyl followed by the unequal growth rates of the root and the hypocotyl. In the present study it was found that the knee begins to straighten out some seven or eight days following germination, rather than the eight to ten days reported by Hayward (1938). The cotyledon measures about seven rather than one centimeter as reported by Hayward (1938) and the root only two or three rather than ten centimeters, at the end of germination.

2. Standard Procedure

Following transfer of the seedlings to the culture solutions, a distinct swelling is observed a short distance

above the root-cap on most roots (Figure 2, a). This swelling is attributed to differences in growth rate between the surface cells and the underlying cell layers of the root apex. It appears that the apical cells at the time of transfer to the treatment conditions do not increase in length as much as normally but instead increase radially. This phenomenon is of particular interest in that the swelling is frequently devoid of an epidermis, the differentiated hypodermis taking over the role of the surface layer of cells. Frequently, the epidermis is not completely present but is absent in patches. Whatever the cause for the formation of this swelling, it is a constant feature in roots grown in all the various culture solutions. Following this initial period of swelling, the root soon resumes its normal rate of growth and diameter (Figure 2, b).

Occasionally, when a root is left for more than ten days in the culture solution, an adventitious root develops from the region of the cotyledonary plate. This root is always wider than the seedling root, and once initiated grows at a much faster rate, often reaching the same length in three days as the seedling or primary root had grown in two weeks (Figure 2, c).

In the thousands of roots examined, only once was a secondary lateral root observed on the seedling root (Figure 2, d).

FIGURE 2

Diagrams showing development of seedling root under experimental conditions.

a. seedling root shortly after transfer to the culture solution. X 15

b. seedling root after normal growth has resumed in the culture solution. X 15

c. onion seedling. X 1

d. onion seedling root showing a secondary root. X 15

FIGURE 3

Epidermal cells of onion seedling root grown in tap water showing slight apical protrusions. X 450

FIGURE 2

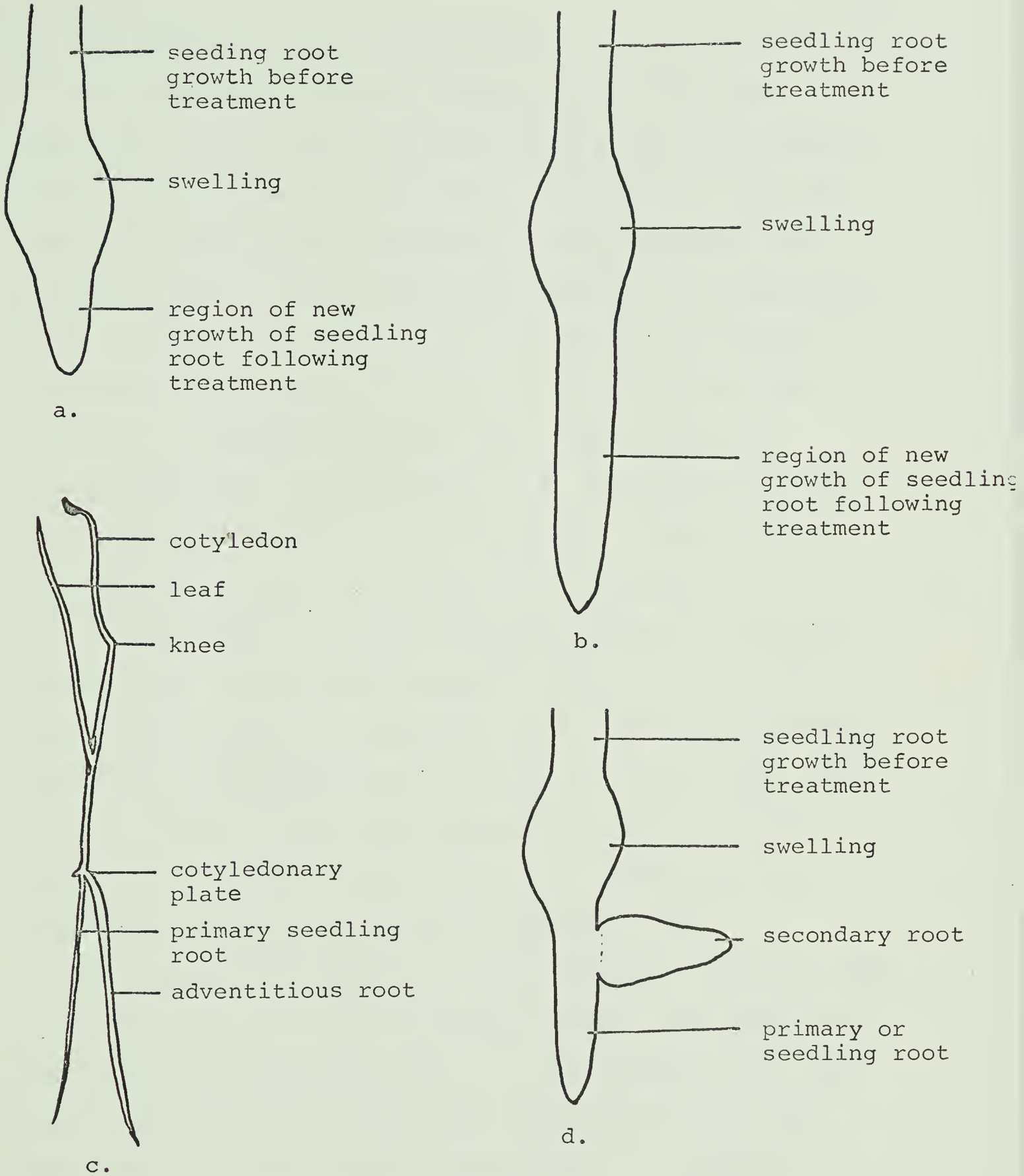


FIGURE 3

B. The Anatomy of the Seedling Root.

The epidermis of onion seedling roots shows great variation from one root to another root in the same culture solution, and often from one place to another on the same root. In spite of this variation of cell shape and size two things can be said of the onion seedling root epidermis. First, the epidermis does not form root hairs in neutral tap water, and second, the epidermis is undifferentiated (Figure 4). Although seedling roots are hairless, in neutral tap water, the epidermal cells frequently develop very short apical protrusions (Figure 3). These apical protrusions are not to be confused with papilla.

Another layer of cells worthy of mention is the hypodermal layer. As its name suggests, this layer of cells lies directly under the epidermis, and despite considerable variation is differentiated into long and short cells (Figure 5). In most cases, this disparity in size is very obvious, but in other cases it is less obvious, and in still other cases it is scarcely obvious at all.

In roots with a differentiated epidermis (Cormack, 1935) it is the short cells that normally produce root hairs while the long cells remain hairless. In the present study, the short hypodermal cells were never observed to form root-hairs, even in those special cases where the epidermis is seen to be torn away or absent. Also, on roots grown in culture solutions which stimulated the epidermal cells to form hairs, no correlation could be observed between the

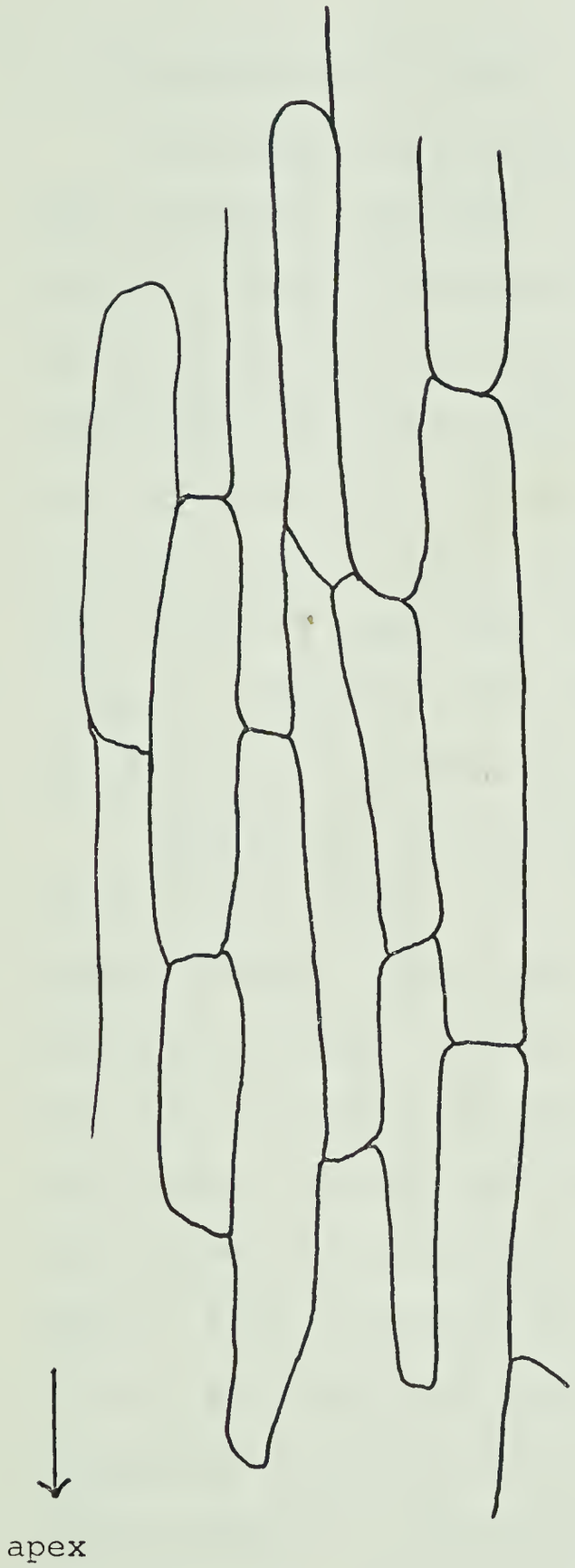
FIGURE 4

Optical section of epidermis of onion seedling root showing the undifferentiated, hairless cells. X 450

FIGURE 5

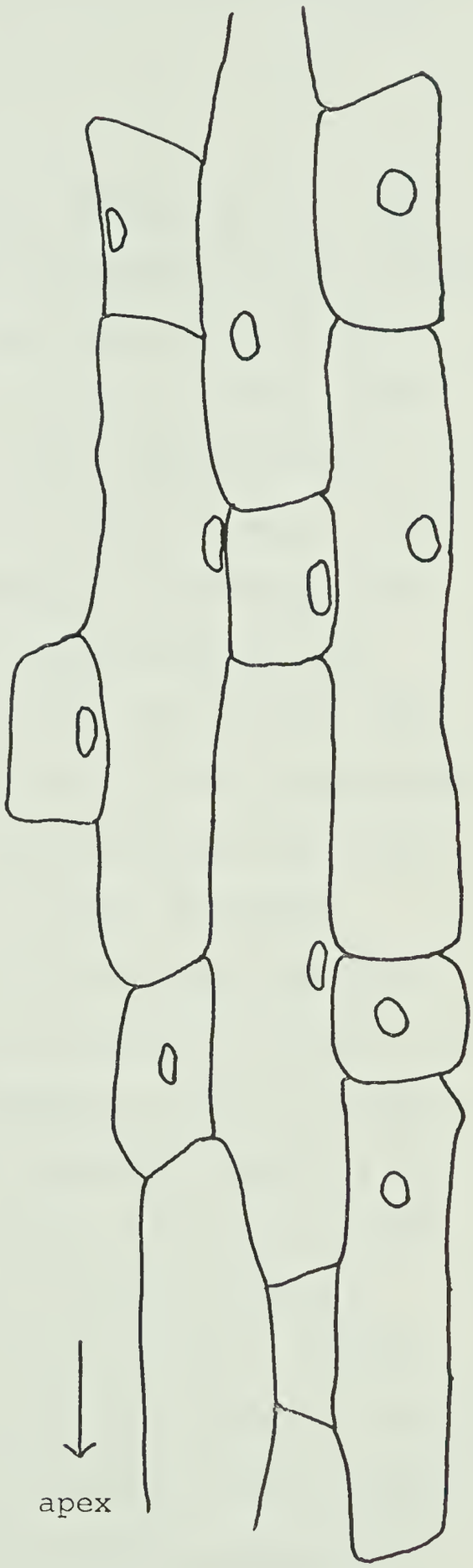
Optical section of the hypodermal layer lying immediately below the epidermal layer shown in Figure 4. X 450

FIGURE 4



Epidermis

FIGURE 5



Hypodermis

hair forming cells of the epidermis and the short hypodermal cells lying directly underneath.

C. Microchemical Studies

The results of the histochemical studies of the roots were extremely revealing. In the microchemical tests, roots grown by three different methods were used. In all the tests use was made of roots grown in water by the method previously described in Methods A, roots grown between two layers of moist filter paper, and roots grown in sand. In every test no significant observable differences were seen in roots grown under the three formerly described methods. Therefore, the chemical composition of the roots would seem to be basically the same in all three conditions.

Testing for the presence of cellulose using the zinc-chlor-iodine method (Jensen, 1962) and the IKI-H_2SO_4 method (Jensen, 1962) give essentially the same results. Fresh roots grown in water, sand, or between two sheets of moist filter paper give negative results. The root hairs themselves, and the epidermal, and hypodermal layers are all yellow even just above the meristem. The only places where a faint purple color is seen is in the vascular tissue, and rarely in the inner cortical layers. Worthy of special attention is the occurrence of a granular material which appears as tiny projections outside the outer epidermal cell wall. This yellow staining granular material is on all the epidermal cell walls. Treatment of the roots

in Xylene for a few days gives much the same results as fresh roots except that in some places where the epidermal cells are very, very long, they acquired a very faint purple color.

The most valuable information was obtained from roots which had been stored in Xylene for one month. The epidermal, hypodermal, papillae, and root-hair cell walls all stain positively. The root hairs, which are often bulbous and wavy, are seen to have quite thick walls right out to the tip. The short cells of the hypodermis are more densely stained than the long cells but it is unknown if this is because they have thicker walls or contain less suberin. Also worthy of note here is that even after one month in xylene, roots treated with zinc-chlor-iodine give a positive reaction, but the epidermis is marked with yellow strips. The root hairs, which are often distorted, are also stained irregularily having both blue and yellow areas of cell wall on the same hair. Cross sections tested for cellulose following one month in xylene give a positive cellulose test. The root cap cells stained deeply but the meristematic cell walls are very thin and appear to contain very little cellulose.

Sudan IV definitely indicates the presence of fatty substances. Roots from water, sand, and moist filter paper all give about the same results. The epidermal and hypodermal cell walls stain red. The radial walls of the hypodermis stain darker than the tangential wall. There is

no observable difference between the staining of the long and short cells. The end walls of both the epidermal and hypodermal cells stain very deeply. Sections of the material reveal that fatty substances are present to a certain extent in the end walls of the cortical cells also. The meristematic cells contain a great deal of fatty materials. However, the cell walls in and near the meristem have little or no fats. In the mature region the inner cortical cells stain somewhat, and the hypodermal and epidermal cell walls, which are very thick, stain deeply. The epidermis has on its outer-most wall a positively staining rough deposit which is as thick as the cell wall or thicker.

Cold chromic acid was used to determine the location of the waxes, cutin, and suberin. Fresh roots treated with chromic acid dissolve almost completely away leaving only parts of the xylem, and the hypodermis, and the epidermis. In the hypodermis there is no observable difference between the long and the short cells. When the roots are treated for a long time with xylene and then treated with chromic acid, they dissolve completely leaving nothing at all. Sections reveal that even in very young tissues the epidermal and hypodermal layers have waxes in the cell wall, as does the xylem. The center metaxylem vessels can still have cytoplasm but the chromic acid does not dissolve the cell wall away as it does the cortex. Here the hypodermal cells are not completely suberized, however, because the inner tangential wall is removed leaving

intact only the radial walls and the outer tangential walls. In the mature root after treatment with chromic acid one sees a thick rough cuticle, epidermal, hypodermal, endodermal, and vessel cell walls. Although the cortex is dissolved away, there is seen in cross section, radiating out from the passage cells, portions of cortical cell walls which have not disappeared.

The phloroglucin-HCl test on fresh roots is negative, but on roots kept in xylene for a very long time it is positive. The triarch xylem is found to have very little lignin and the rest of the tissues have none.

Using the ruthenium red technique described by Johansen (1940) to test for pectic substances, the dye turns the meristem a very deep red. Further back the cell walls are pink. Those epidermal cells which do not form hairs or papillae are very light pink while those that do are definitely pink. The hairs themselves are pink or red right to the tip of the hair. It is interesting to note that those hairs closer to the tip are redder than the older more basal ones. When the roots are treated with hydrochloric acid and then stained, the meristem is pink, and the epidermal cell walls near the meristem are pink but further back they are colorless unless the cell produces a hair in which case frequently the epidermal cell wall is pink. In general, the hairs themselves are stained deeply right to the tip with the base of the hair often being very red.

If, following treatment with hydrochloric acid the roots are treated with ammonium hydroxide and stained, the meristem and the epidermal cell walls are very faint pink or colorless. However, the hypodermal layer is frequently still pink, especially the short cells which may be quite red. There is a tendency for roots grown in water, compared to those from sand or moist filter paper, to stain more readily initially, and following the hydrochloric acid treatment, and to stain not as much following the ammonium hydroxide treatment. Roots treated with xylene give much the same results as fresh roots except that the results are even more pronounced. The meristem cell walls are very, very densely stained by ruthenium red. The hypodermal, epidermal, and root hair walls are also well stained.
Those roots treated with hydrochloric acid and then stained result in colorless or very faint pink meristematic cell walls, and pink or red epidermal cell walls. The hypodermal cell walls also stain and often the short cell walls are more deeply stained than those of the long cells. The root hair cell walls are sometimes stained.
Roots stored in xylene for a long time and treated with hydrochloric acid and ammonium hydroxide, and then stained with ruthenium red are virtually colorless. These results indicate that ruthenium red is staining pectic compounds exclusively.

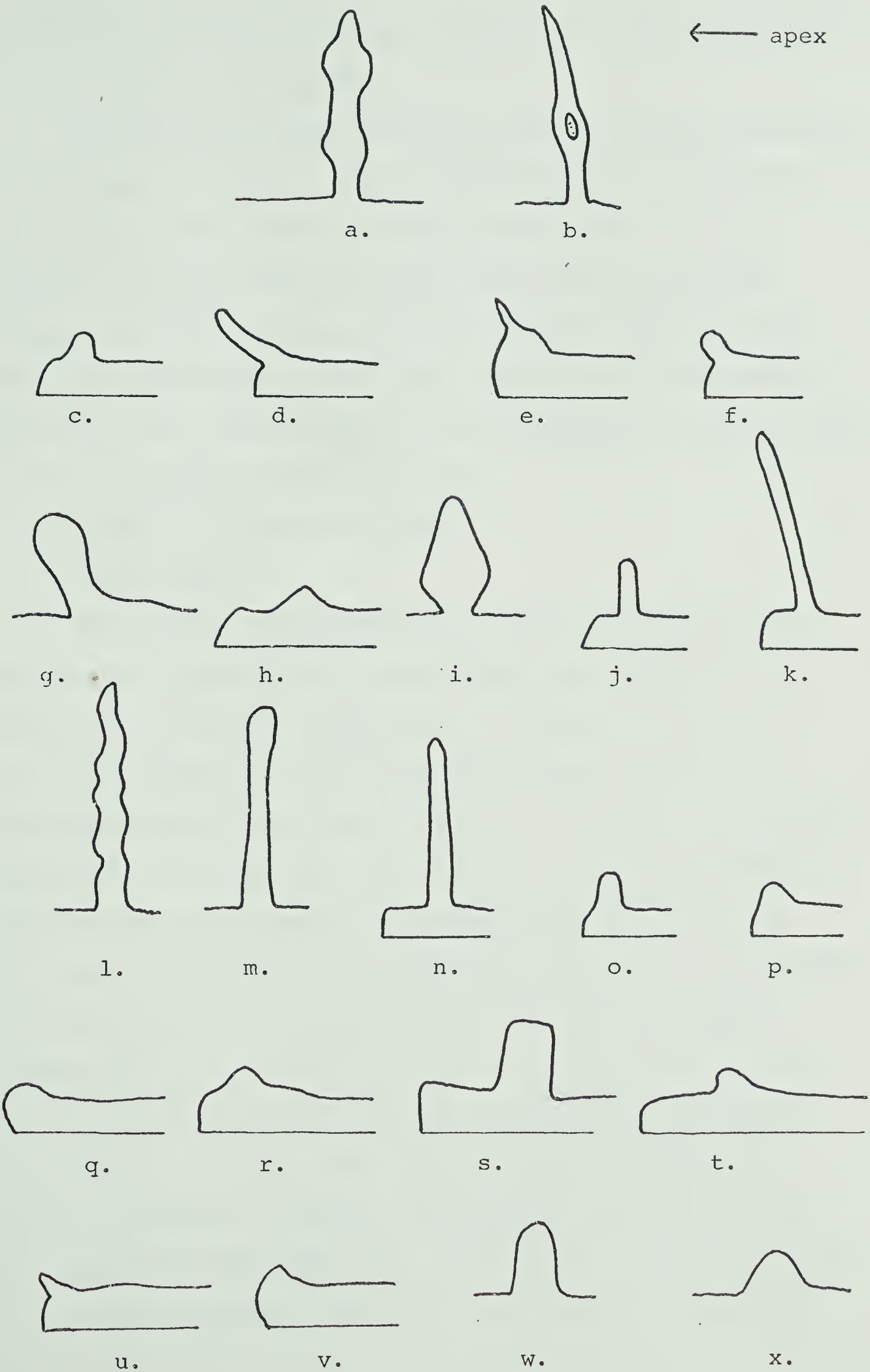
FIGURE 6

Drawings of epidermal cells of onion seedling roots showing the development of root hairs, papillae, and swellings occurring in tap water at various pH's. X 450

a. pH 5
b. pH 5
c. pH 5
d. pH 5
e. pH 5
f. pH 5
g. pH 5.5
h. pH 5.5
i. pH 5.5
j. pH 5 - 6
k. pH 5 - 6
l. pH 6
m. pH 6
n. pH 7
o. pH 7
p. pH 7
q. pH 7
r. pH 8
s. pH 8.5
t. pH 8.5
u. pH 9
v. pH 9
w. pH 9
x. pH 9

Control is shown in Figure 3.

FIGURE 6



D. Effects of Various Culture Solutions on Root Growth and on Root Hair Development.

The growth of onion seedling roots in aqueous solutions is extremely variable. This observation is in agreement with that of Mer (1884) for onion bulb roots. In the present study of onion seedling roots great variation occurs from root to root, in roots growing under exactly the same conditions in the same beaker of the same culture solution, and from one part of the epidermis to another part of the epidermis of the same root.

1. Effect of Calcium and pH.

a. Tap Water

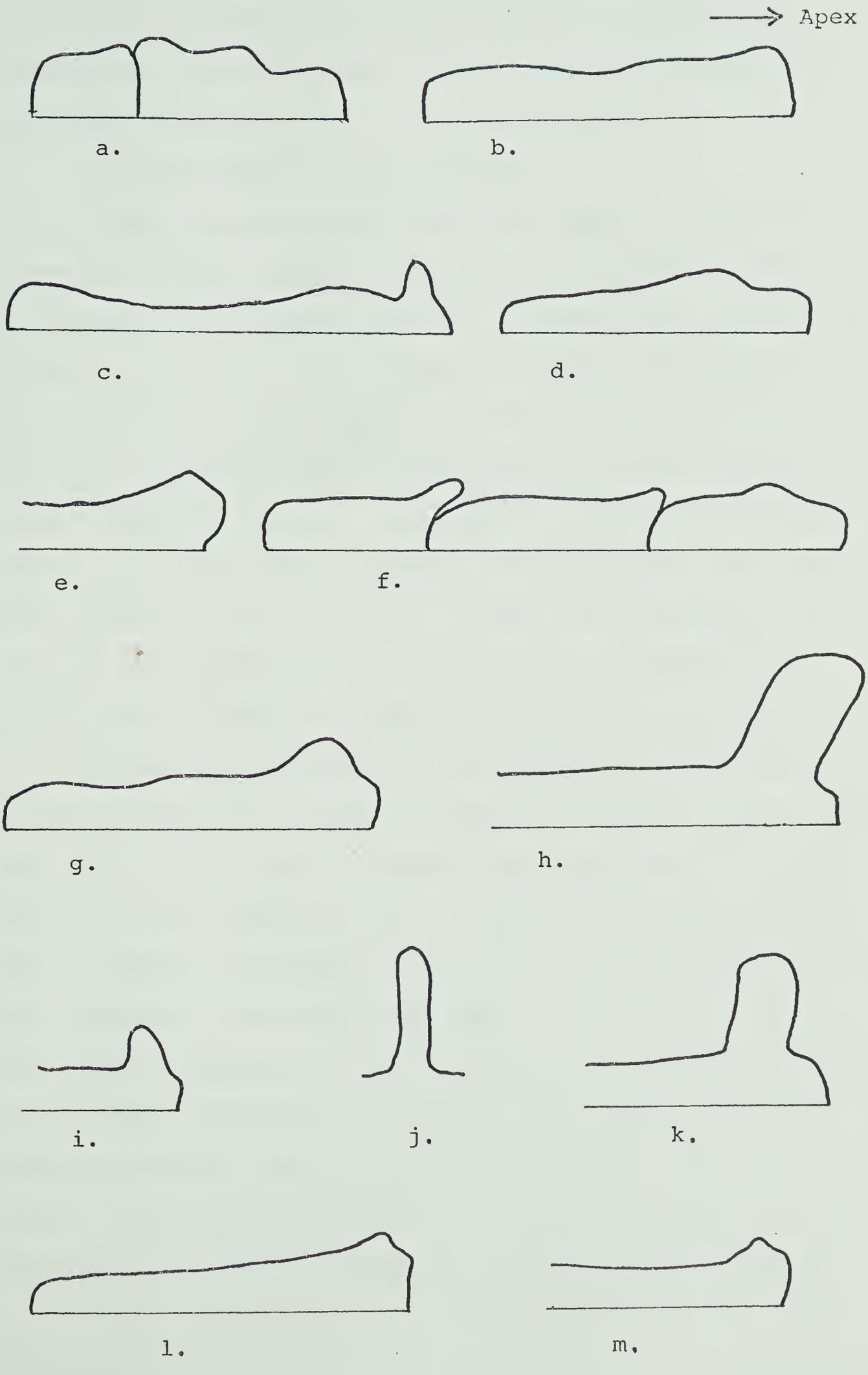
The results of experiments to test the effect of pH on root hair development on seedling roots grown in tap water may be briefly summarized as follows. At neutrality the seedling roots are hairless except for the very rare papilla and root hair (Figure 6, n,o,p,q). Increased acidity of the tap water stimulates root hair development, with maximum development occurring at pH of 5.0 to 6.0. The hairs vary greatly in length and shape and occasionally they are swollen in various places along their length (Figure 6, a, b, c, d, e, f, g, h, i, j, k, l, m). At a pH of 4.5 the roots fail to grow although the seedlings form leaves. On the other hand, increased alkalinity tends to decrease root-hair development, and to decrease the length of the hairs. At a pH of 8.0 - 9.0 some of the roots show epidermal cells with swollen ends, simulating

FIGURE 7

Drawings of epidermal cells of onion seedling roots showing the development of root hairs, papillae, and swellings occurring in saturated calcium sulphate solutions at various pH's. X 450

a. pH 4.5
b. pH 4.5
c. pH 4.5
d. pH 4.5
e. pH 4.5
f. pH 5
g. pH 5
h. pH 6
i. pH 6
j. pH 6
k. pH 6
l. pH 7
m. pH 8

FIGURE 7



short hairs or papillae, other roots show a scattering of papillae, and still other show a sparce development of short hairs (Figure 6, r, s, t, u, v, w, x).

b. Saturated Calcium Sulphate

Root growth and root-hair development is poorer in saturated calcium sulphate solutions as compared to that in tap water. The general effect of adding some calcium to the aqueous solution is to depress root-hair development. In neutral and slightly alkaline solutions the epidermal cells are extremely long and both hairs and papillae are absent (Figure 7, l, m). In slightly acid solutions root-hairs occur, but they are usually abnormally wide and very short (Figure 7, h, i, j, k). In more acid solutions, at about pH 5.0 hairs cease to form and the epidermal cells are frequently swollen (Figure 7, a, b, c, d, e, f, g).

The general conclusion to be reached from the results of these experiments is that calcium in greater amounts than that found in tap water depresses the development of root-hairs on onion seedling roots. Slightly alkaline solutions have a similar depressing effect while slightly acid solutions stimulate root-hair development. These observations are directly opposed to those reported for the development of root hairs on *Brassica* seedling roots grown in aqueous calcium solutions and at varying pH (Cormack, 1935) and suggest important differences in the chemical nature of the epidermal cell walls of *Brassica* and onion seedling roots.

In this connection, it is worth mentioning that hypo-

FIGURE 8

Drawings of epidermal cells of onion seedling roots showing the development of root hairs, papillae and swellings occurring in solutions of various concentrations of Na_2EDTA made up in distilled water. X 450

a. $10^{-3.5}$ M Na_2EDTA

b. $10^{-4.5}$ M Na_2EDTA

c. $10^{-4.5}$ M Na_2EDTA

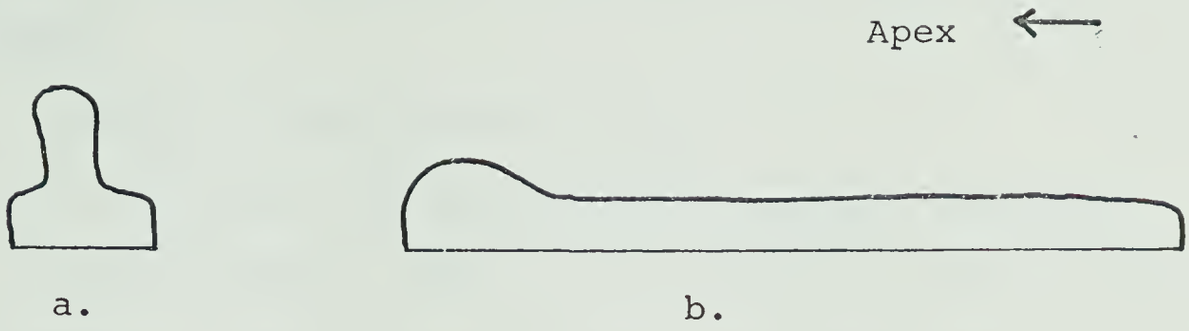
d. $10^{-5.5}$ M Na_2EDTA

e. $10^{-5.5}$ M Na_2EDTA

f. $10^{-5.5}$ M Na_2EDTA

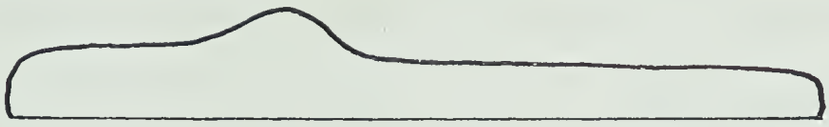
g. $10^{-6.5}$ M Na_2EDTA

FIGURE 8



a.

b.

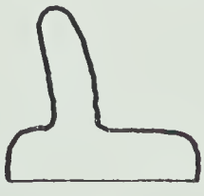


c.

d.

e.

f.



g.

dermal layer of onion seedling roots is much more heavily suberized in the alkaline solutions than in the acid solutions.

2. Effect of Calcium Deficiency

a. Disodium ethylenediaminetetracetate (Na_2EDTA)

In the present study, it was found possible to grow onion seedling roots in solutions of Na_2EDTA made up either with distilled water, tap water, or with a saturated solution of calcium sulphate. The best root growth occurred in the latter solution and the poorest in the solutions of Na_2EDTA in distilled water. In all these experiments with Na_2EDTA, the pH of the solutions was maintained at 7.0. Root hair development is poor at all concentrations of Na_2EDTA but is somewhat better at lower concentrations than in solutions of higher concentration. Swelling of the epidermal cells occurs to some extent in all concentrations of Na_2EDTA, but is more striking in solutions of higher concentration.

i. Na_2EDTA in distilled water.

In solutions of Na_2EDTA in distilled water marked abnormalities take place in the epidermis. In some cases the epidermal cells are curled up at the apical end, and in others the epidermal cells are variously swollen, and in still others they form papillae or short hairs, especially when the epidermal cells are short (Figure 8, a, b, c, d, e, f, g).

FIGURE 9

Drawings of epidermal cells of onion seedling roots showing the development of root hairs, papillae, and swellings occurring in solutions of various concentrations of Na_2EDTA made up in tap water. X 450

a. $10^{-3.5}$ M Na_2EDTA

b. $10^{-4.5}$ M Na_2EDTA

c. $10^{-5.5}$ M Na_2EDTA

d. $10^{-5.5}$ M Na_2EDTA

e. $10^{-5.5}$ M Na_2EDTA

f. $10^{-5.5}$ M Na_2EDTA

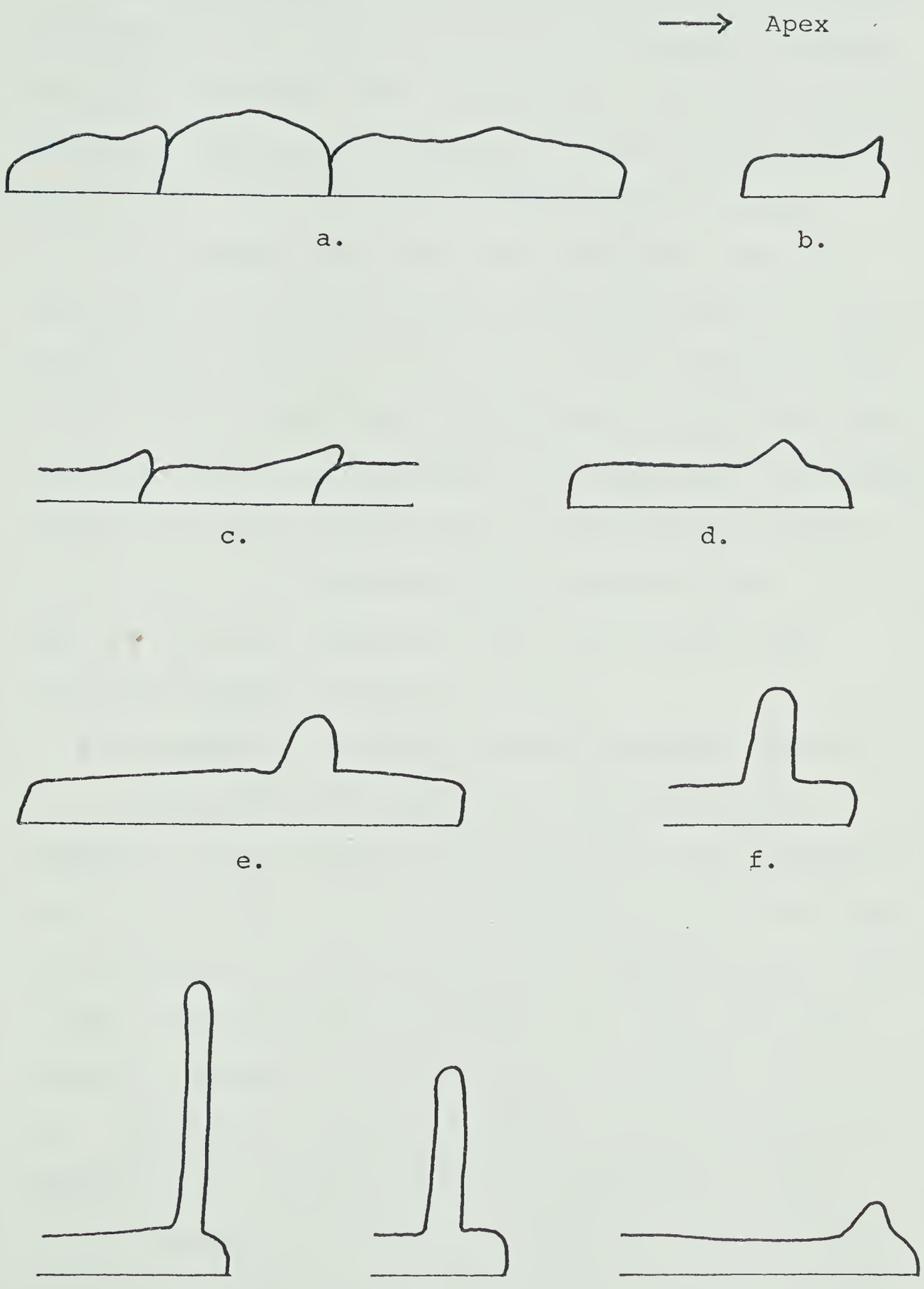
g. $10^{-6.5}$ M Na_2EDTA

h. $10^{-6.5}$ M Na_2EDTA

i. $10^{-6.5}$ M Na_2EDTA

Control is shown in Figure 3.

FIGURE 9



ii. Na_2EDTA in tap water.

The same thing is true of roots grown in solutions of Na_2EDTA in tap water at all concentrations ranging from $10^{-3 \cdot 5}M$ to $10^{-6 \cdot 5}M$. In general, variously swollen epidermal cells occur frequently in all concentrations (Figure 9a). At the lower Na_2EDTA concentration, $10^{-6 \cdot 5}M$, papillae and hairs are relatively numerous. These hairs are usually short but occasionally they are fairly long (Figure 9, g, h, i). At the concentration of $10^{-5 \cdot 5}M$ Na_2EDTA, the root epidermis is much the same as at the lower concentration, but when hairs are formed these tend to be shorter and very much wider (Figure 9 c, d, e, f). At higher Na_2EDTA concentrations, $10^{-4 \cdot 5}M$ and $10^{-3 \cdot 5}M$, the epidermal cells are hairless and variously swollen (Figure 9, a, b).

iii.Na_2EDTA in saturated calcium sulphate solution.

In solutions of Na_2EDTA in saturated calcium sulphate, onion seedling roots grow best at a concentration of $10^{-5 \cdot 5}M$. Here, the epidermal cells are long, and hairless with undulating walls (Figure 10, e). At a lower concentration of Na_2EDTA, $10^{-6 \cdot 5}M$, hairs are commonly produced as well as papillae (Figure 10, f, g, h). The general effects of higher concentrations of Na_2EDTA is to inhibit root hair development. At a concentration of $10^{-4 \cdot 5}M$, the epidermal cells tend to be long and they are either hairless or rarely produce small papillae-like protrusions (Figure 10 b, c). At a

FIGURE 10

Drawings of epidermal cells of onion seedling roots showing the development of root hairs, papillae, and swellings occurring in solutions of various concentrations of Na_2EDTA made up in a solution of saturated calcium sulphate.

X 450

a. $10^{-3 \cdot 5}$M Na_2EDTA

b. $10^{-4 \cdot 5}$M Na_2EDTA

c. $10^{-4 \cdot 5}$M Na_2EDTA

d. $10^{-5 \cdot 5}$M Na_2EDTA

e. $10^{-5 \cdot 5}$M Na_2EDTA

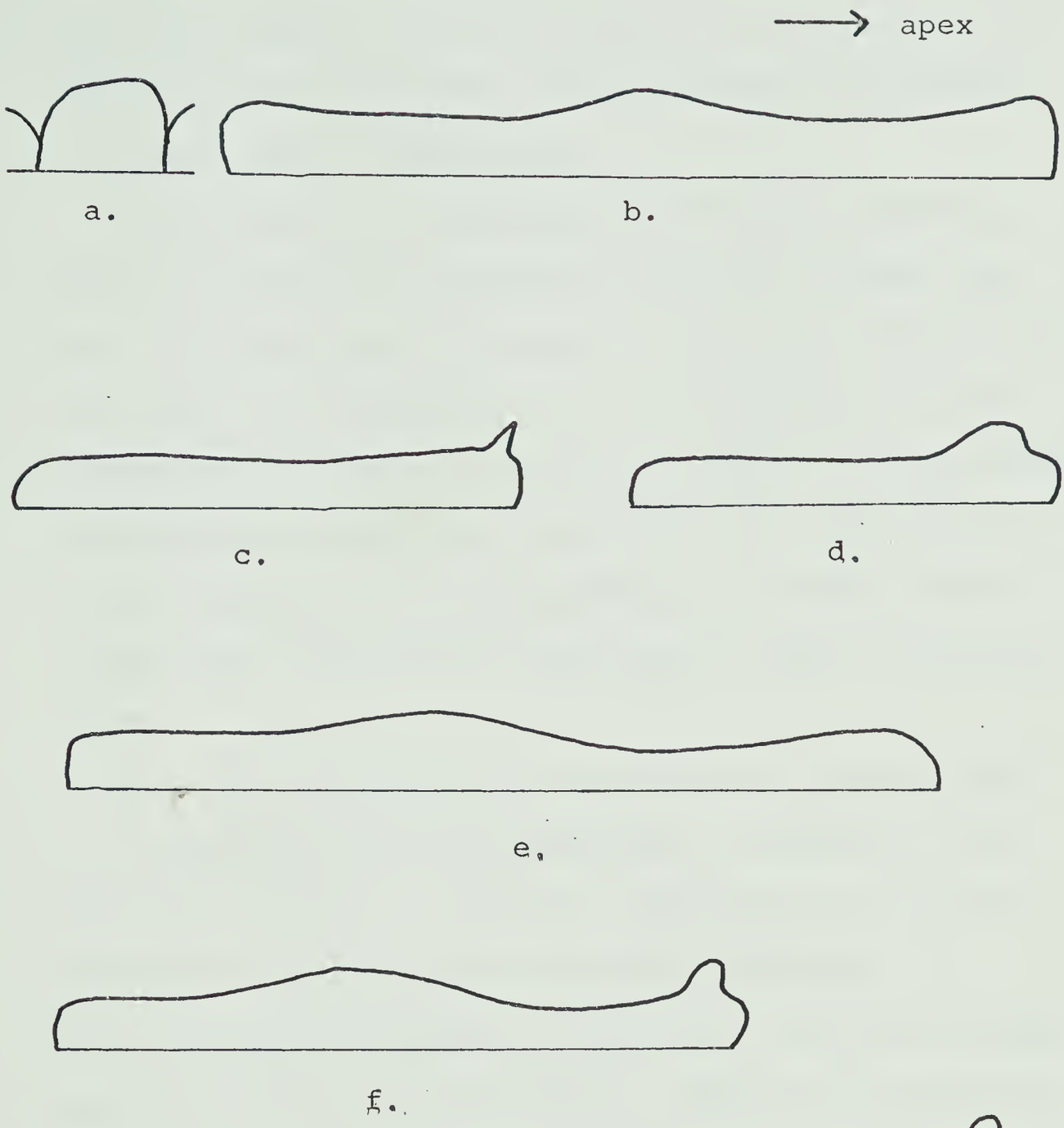
f. $10^{-6 \cdot 5}$M Na_2EDTA

g. $10^{-6 \cdot 5}$M Na_2EDTA

h. $10^{-6 \cdot 5}$M Na_2EDTA

Control is shown in Figure 7 1.

FIGURE 10



still higher concentration, $10^{-3 \cdot 5}$M, there is little or no root growth. The poor growth of the roots in these solutions of higher concentration is attributed to marked calcium deficiency.

The results of experiments with onion seedling roots in solutions of EDTA may be briefly summarized. Root hair development is poor at all concentrations of EDTA, but it is somewhat better in solutions of lower concentration than in solutions of higher concentration. Swelling of the hairless epidermal cells takes place to some extent in all concentrations of EDTA, but it is much more striking in solutions of higher concentrations.

iv. $10^{-4 \cdot 5}$M Na_2EDTA in tap water at various pH's.

The next series of experiments designed to test the effect of pH on root hair development in calcium deficient solutions may be briefly described. A $10^{-4 \cdot 5}$M Na_2EDTA solution in tap water was used throughout this series of experiments. The pH was varied by means of phosphate buffers. In general, the development of root hairs or papilae at all pH's in this series was sparce.

The general effect of both alkaline and neutral solutions is to inhibit the development of root hairs and to favour longitudinal elongation of the epidermal cells and the production of a wide variety of papillae and short projections (Figure 11 g, h, i, j, k, l). At

FIGURE 11

Drawings of epidermal cells of onion seedling roots showing the development of root hairs, and swellings occurring in solutions of $10^{-4.5}$ M Na_2EDTA in tap water at various pH's. X 450

a. pH 4.5

b. pH 5

c. pH 5

d. pH 6

e. pH 6

f. pH 6

g. pH 7

h. pH 7

i. pH 8.5

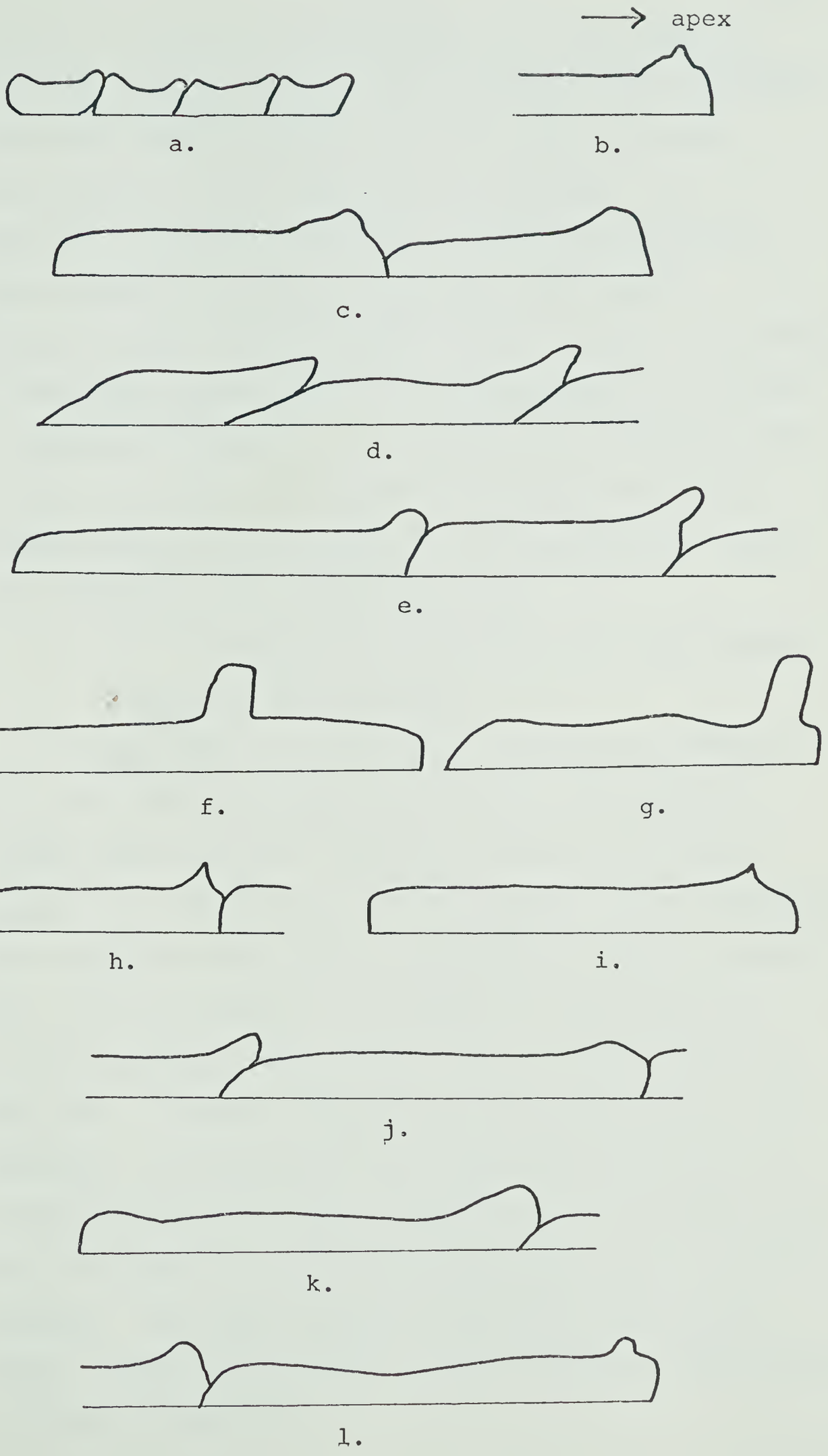
j. pH 8.5

k. pH 9

l. pH 9

Control is shown in Figure 9 b.

FIGURE 11



a pH of 8.5 and 9.0 the epidermal cells are long and hairless, and the walls of both epidermal and hypodermal cells are heavily coated with a wax-like substance. Occasionally at this same pH, the epidermal layer is found to be torn and ruptured, leaving the hypodermal layer exposed at intervals over the root surface. In general, weakly acid solutions tend to hinder the growth of the roots and to favour the development of a variety of papillae (Figure 11, b, c, d, e, f). Increasing the acidity to a pH of 4.5 results in little or no root growth and in the development of extremely short, hairless epidermal cells (Figure 11, a).

v. $10^{-4 \cdot 5}$M Na_2EDTA in a saturated calcium sulphate solution at various pH's.

In the series of experiments to test the effect of pH on root hair development in a $10^{-4 \cdot 5}$M solution of Na_2EDTA made up with a saturated solution of calcium sulphate the results are much the same as those obtained with Na_2EDTA in tap water. In general, there is greater over all root growth in the series of experiments in calcium sulphate than in the series of experiments in tap water. Another difference in the two series is the tendency, in neutral and acid solutions especially, to produce roots with longer epidermal cells and fewer root hairs or papillae in the solutions made up with calcium sulphate. The great range in epidermal cell length, shape, and form that occurs in this series of experiments is shown in Figure 12.

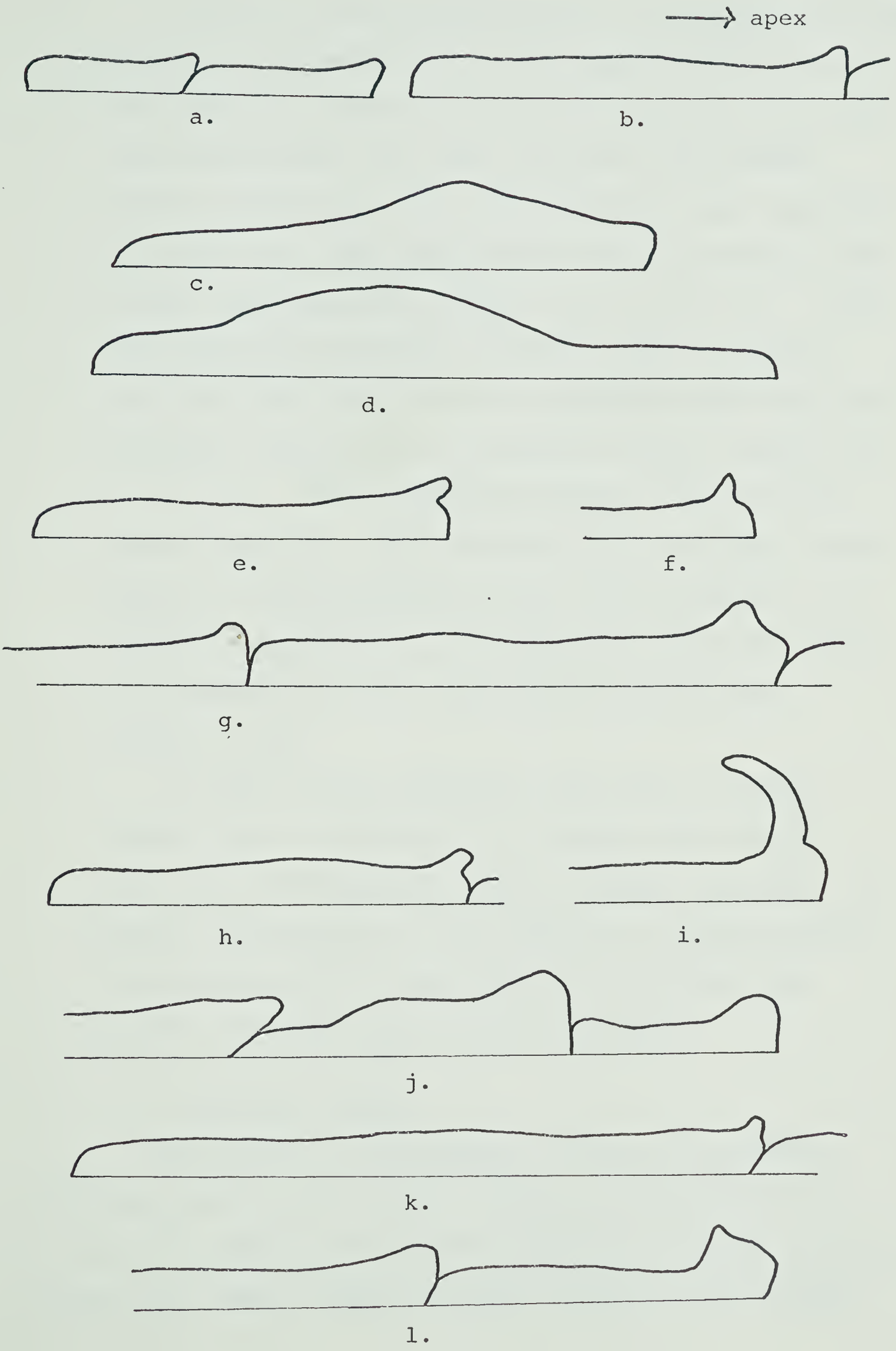
FIGURE 12

Drawings of epidermal cells of onion seedling roots showing the development of root hairs, papillae, and swellings occurring in solutions of $10^{-4.5}$ M Na_2EDTA in a solution of saturated calcium sulphate at various pH's. X 450

a. pH 4.5
b. pH 5
c. pH 6
d. pH 6
e. pH 6
f. pH 7
g. pH 7
h. pH 7
i. pH 7
j. pH 8
k. pH 8
l. pH 9

Controls are shown in Figure 10, b and c.

FIGURE 12



At pH 9.0 the epidermal cells are generally hairless but very occasionally form small papillae (Figure 12,1). When the pH is lowered to 8.0 most epidermal cells are long and hairless, but some are short and somewhat swollen (Figure 12,j, k). At neutrality most epidermal cells are very long and hairless but occasionally they produce small papillae (Figure 12, f, g, h, i). On the other hand acid solutions of pH 6.0 and 5.0 produce roots with very long hairless epidermal cells which are frequently undulating or variously swollen (Figure 12, b, c, d, e). These extremely swollen cells are most common at pH 6.0. These slightly acid solutions rarely result in the development of very small papillae. At a still lower pH of 4.5, the epidermal cells are short and produce very small papillae-like projections (Figure 12, a).

At this pH the epidermal cells have so little cuticle that it dissolves on treatment with chromic acid, and the hypodermal layer is poorly suberized. In general cutinization of the epidermis and suberization of the hypodermis is much greater in alkaline and neutral solutions than in acid solutions.

b. Ammonium oxalate

The results of experiments carried out by adding increasing amounts of ammonium oxalate to distilled water, to tap water, and to a saturated solution of calcium sulphate are much the same as those described above for the Na_2EDTA

FIGURE 13

Drawings of epidermal cells of onion seedling roots showing the development of root hairs, papillae, and swellings occurring in solutions of various concentrations of ammonium oxalate made up in distilled water. X 450

a. 10^{-3} M ammonium oxalate

b. 10^{-3} M ammonium oxalate

c. 10^{-3} M ammonium oxalate

d. $10^{-3.5}$ M ammonium oxalate

e. $10^{-3.5}$ M ammonium oxalate

f. $10^{-3.5}$ M ammonium oxalate

g. $10^{-3.5}$ M ammonium oxalate

h. 10^{-4} M ammonium oxalate

i. 10^{-4} M ammonium oxalate

j. 10^{-4} M ammonium oxalate

k. 10^{-4} M ammonium oxalate

l. 10^{-5} M ammonium oxalate

m. 10^{-5} M ammonium oxalate

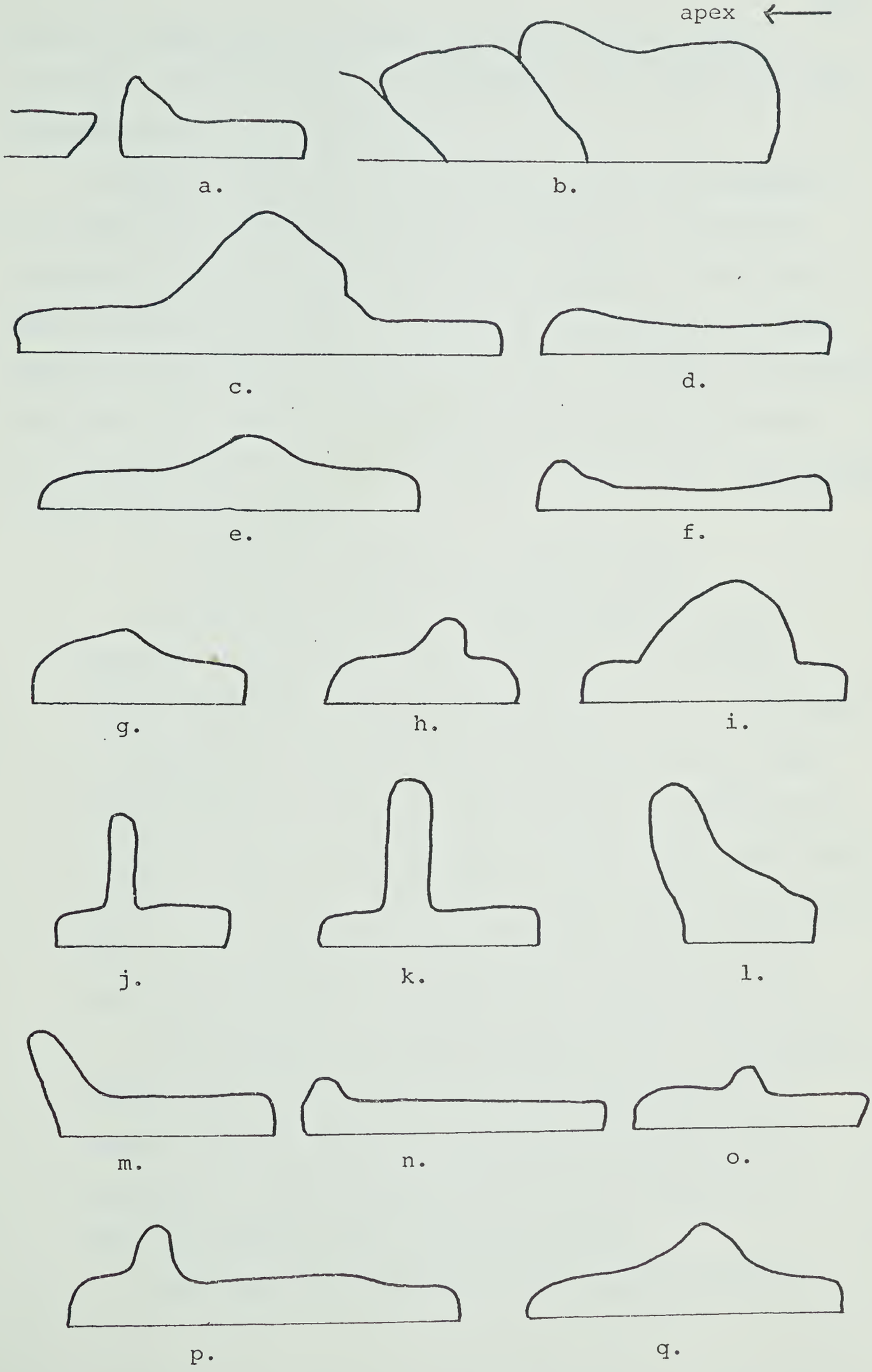
n. 10^{-5} M ammonium oxalate

o. 10^{-5} M ammonium oxalate

p. 10^{-5} M ammonium oxalate

q. 10^{-5} M ammonium oxalate

FIGURE 13



series of experiments although possibly they are more striking. In this series of experiments, all solutions were maintained at a pH of about 7.0.

In general both root growth and root hair development are better when ammonium oxalate is added to a saturated solution of calcium sulphate than when added to either tap water or distilled water. Maximum root growth in all three series of experiments occurs at $10^{-3 \cdot 5}$M. At higher concentrations the toxic effects of ammonium oxalate are evidenced by a decrease in the growth of the root and by the suppression of root hair development.

i. Ammonium oxalate in distilled water.

The general effect of ammonium oxalate in all concentrations from 10^{-3}M to $10^{-7 \cdot 5}$M is to produce marked abnormalities in the epidermal layer. The degree of abnormality varies from root to root but the shape and arrangement of the epidermal cells are modified to a certain extent in every root. The abnormal appearance of the epidermis of this kind of root, and the concentration of the ammonium oxalate solution at which they most frequently occur is shown in Figures 13 and 14.

The optimum root growth occurs at $10^{-3 \cdot 5}$M ammonium oxalate and is characterized by the development of rather long and frequently swollen epidermal cells. Generally as the oxalate concentration decreases so does the length of the epidermal cells.

Swelling of the epidermal cells occurs at all

FIGURE 14

Drawings of epidermal cells of onion seedling roots showing the development of root hairs, papillae, and swellings occurring in solutions of various concentrations of ammonium oxalate made up in distilled water. X 450

a. 10^{-5} M ammonium oxalate

b. 10^{-5} M ammonium oxalate

c. 10^{-5} M ammonium oxalate

d. $10^{-6 \cdot 5}$ M ammonium oxalate

e. $10^{-6 \cdot 5}$ M ammonium oxalate

f. $10^{-6 \cdot 5}$ M ammonium oxalate

g. $10^{-6 \cdot 5}$ M ammonium oxalate

h. $10^{-6 \cdot 5}$ M ammonium oxalate

i. 10^{-7} M ammonium oxalate

j. 10^{-7} M ammonium oxalate

k. 10^{-7} M ammonium oxalate

l. 10^{-7} M ammonium oxalate

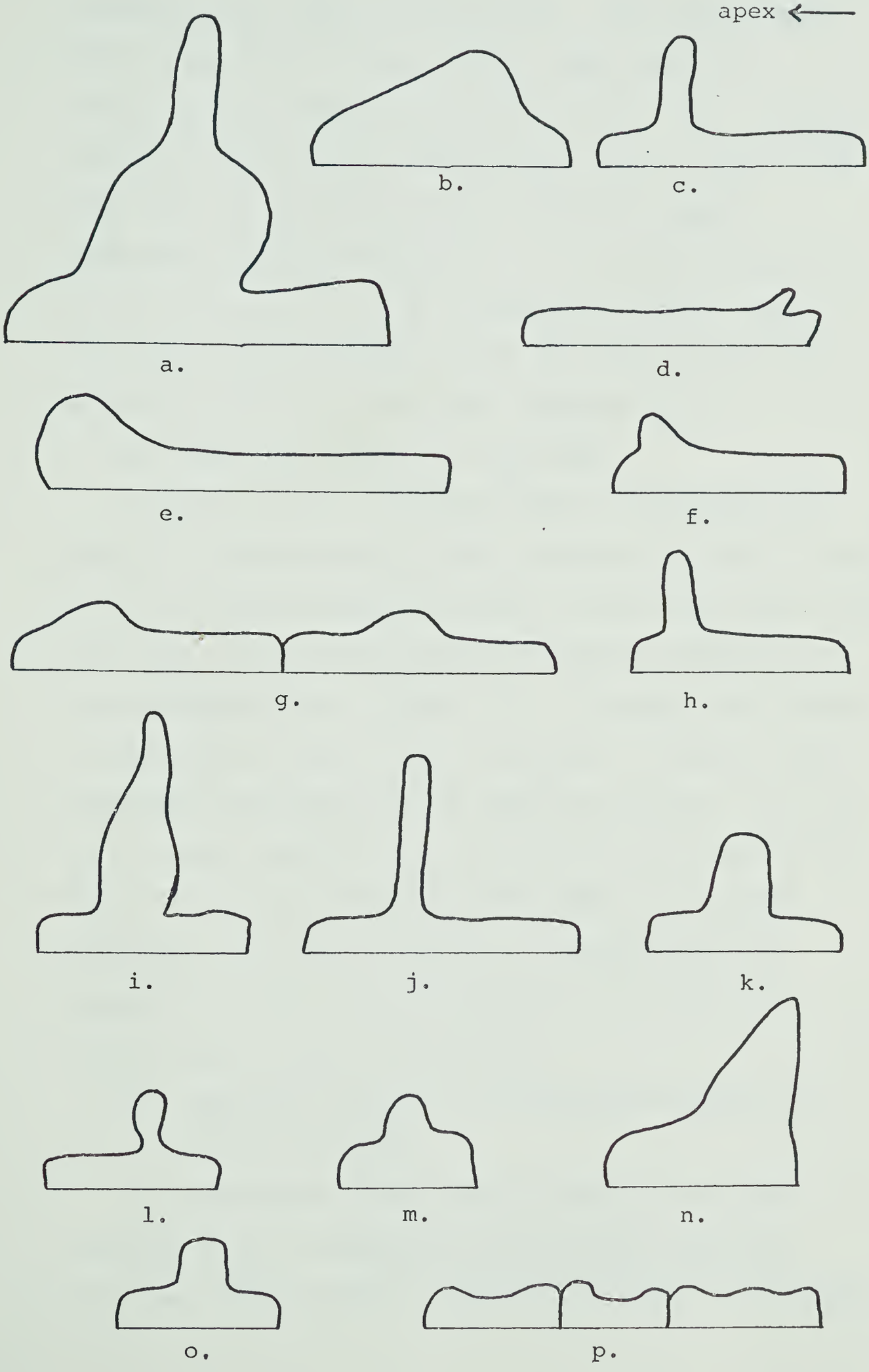
m. 10^{-7} M ammonium oxalate

n. 10^{-7} M ammonium oxalate

o. $10^{-7 \cdot 5}$ M ammonium oxalate

p. $10^{-7 \cdot 5}$ M ammonium oxalate

FIGURE 14



concentrations from 10^{-3}M to 10^{-7}M. The best development of papillae and short hairs takes place in concentrations ranging from 10^{-4}M to $10^{-7 \cdot 5}$M. In many cases, the short hairs and papillae are so wide at the base that the epidermal cells themselves appear abnormally swollen (Figure 13, c, i, q, and Figure 14, a, b, k, m, n, o). At 10^{-3}M ammonium oxalate some of the ends of the epidermal cells are disconnected from adjoining cells in some manner (Figure 13, a).

ii. Ammonium oxalate in tap water.

Growth of onion seedling roots in solutions of ammonium oxalate made up with tap water is much the same as in the series described above in distilled water, and once again it is characterized by marked deformity of the epidermal layer (Figure 15). In general the number of papillae and short hairs increases in density from the lowest concentration of ammonium oxalate, $10^{-7 \cdot 5}$M to a maximum production at a concentration of $10^{-4 \cdot 5}$M. There is also an increase in the number of swollen epidermal cells with increase in concentration of ammonium oxalate and an increase in the number of swollen papillae and short hairs.

iii. Ammonium oxalate in a saturated solution of calcium sulphate.

As mentioned already the best growth of onion seedling roots in ammonium oxalate takes place in solutions made up with a saturated solution of calcium

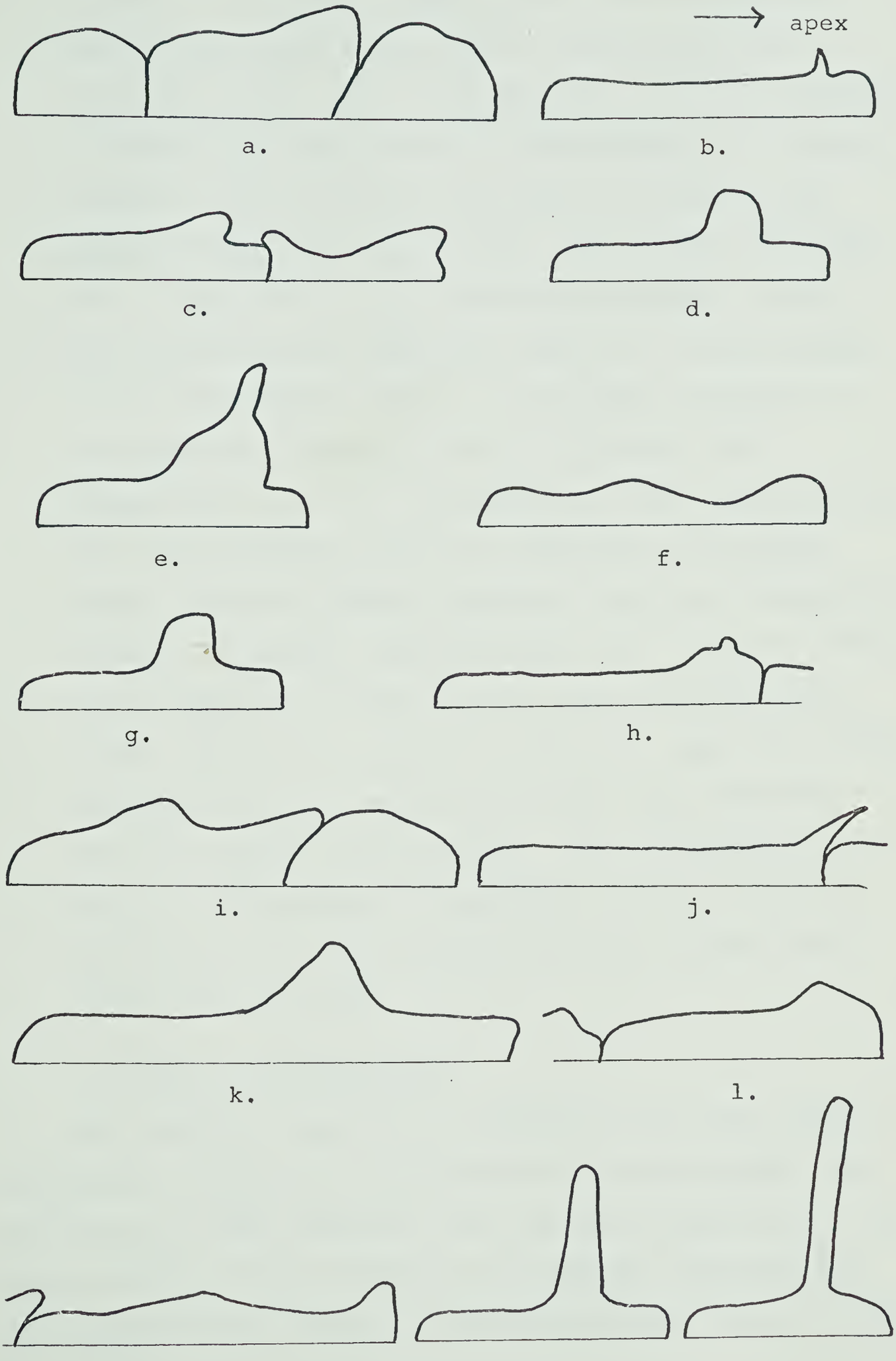
FIGURE 15

Drawings of epidermal cells of onion seedling roots showing the development of root hairs, papillae, and swelling occurring in solutions of various concentrations of ammonium oxalate made up in tap water. X 450

a. $10^{-2.5}$ M ammonium oxalate
b. $10^{-3.5}$ M ammonium oxalate
c. $10^{-3.5}$ M ammonium oxalate
d. $10^{-3.5}$ M ammonium oxalate
e. $10^{-4.5}$ M ammonium oxalate
f. $10^{-4.5}$ M ammonium oxalate
g. $10^{-4.5}$ M ammonium oxalate
h. $10^{-4.5}$ M ammonium oxalate
i. $10^{-5.5}$ M ammonium oxalate
j. $10^{-5.5}$ M ammonium oxalate
k. $10^{-5.5}$ M ammonium oxalate
l. $10^{-5.5}$ M ammonium oxalate
m. $10^{-6.5}$ M ammonium oxalate
n. $10^{-6.5}$ M ammonium oxalate
o. $10^{-7.5}$ M ammonium oxalate

Control is shown in Figure 3.

FIGURE 15



sulphate. In general, papillae and short hairs are sparcely developed at the lower concentrations of ammonium oxalate, $10^{-7 \cdot 5}$M (Figure 16, t, u) but increase in number with the increase in concentration of ammonium oxalate. There is also a noticeable increase in the number of wide, short root hairs, and swollen epidermal cells (Figure 16, r, s). Maximum development of wide short hairs and wide papillae occurs at a concentration of $10^{-5 \cdot 5}$M ammonium oxalate, with marked variation in the number and degree of swollen epidermal cells (Figure 16, m, n, o). In general, at this concentration the longer epidermal cells are hairless and normally shaped, while the shorter epidermal cells are abnormally swollen. At higher concentrations, $10^{-4 \cdot 5}$M and $10^{-3 \cdot 5}$M, hairs become less common but are still wide and short (Figure 16, d, e, f, g, h, i, j, k, l). Again the shorter epidermal cells are swollen while the long epidermal cells are regular, and hairless. At a concentration of about $10^{-2 \cdot 5}$M most of the epidermal cells are swollen and hairless or rarely produce very wide, short hairs (Figure 16, a, b, c).

3. Effect of lipoxygenase.

The results of experiments designed to test the effect of aqueous solutions of lipoxygenase on the development of root hairs on onion seedling roots are most significant. The general effect of the solution is to greatly stimulate the development of root hairs. In this connection, the pH of the

FIGURE 16

Drawings of epidermal cells of onion seedling roots showing the development of root hairs, papillae, and swellings occurring in solutions of various concentrations of ammonium oxalate made up in a solution of saturated calcium sulphate.

X 450 a. $10^{-2.5}$ M ammonium oxalate

b. $10^{-2.5}$ M ammonium oxalate

c. $10^{-2.5}$ M ammonium oxalate

d. $10^{-3.5}$ M ammonium oxalate

e. $10^{-3.5}$ M ammonium oxalate

f. $10^{-3.5}$ M ammonium oxalate

g. $10^{-3.5}$ M ammonium oxalate

h. $10^{-3.5}$ M ammonium oxalate

i. $10^{-4.5}$ M ammonium oxalate

j. $10^{-4.5}$ M ammonium oxalate

k. $10^{-4.5}$ M ammonium oxalate

l. $10^{-4.5}$ M ammonium oxalate

m. $10^{-5.5}$ M ammonium oxalate

n. $10^{-5.5}$ M ammonium oxalate

o. $10^{-5.5}$ M ammonium oxalate

p. $10^{-5.5}$ M ammonium oxalate

q. $10^{-5.5}$ M ammonium oxalate

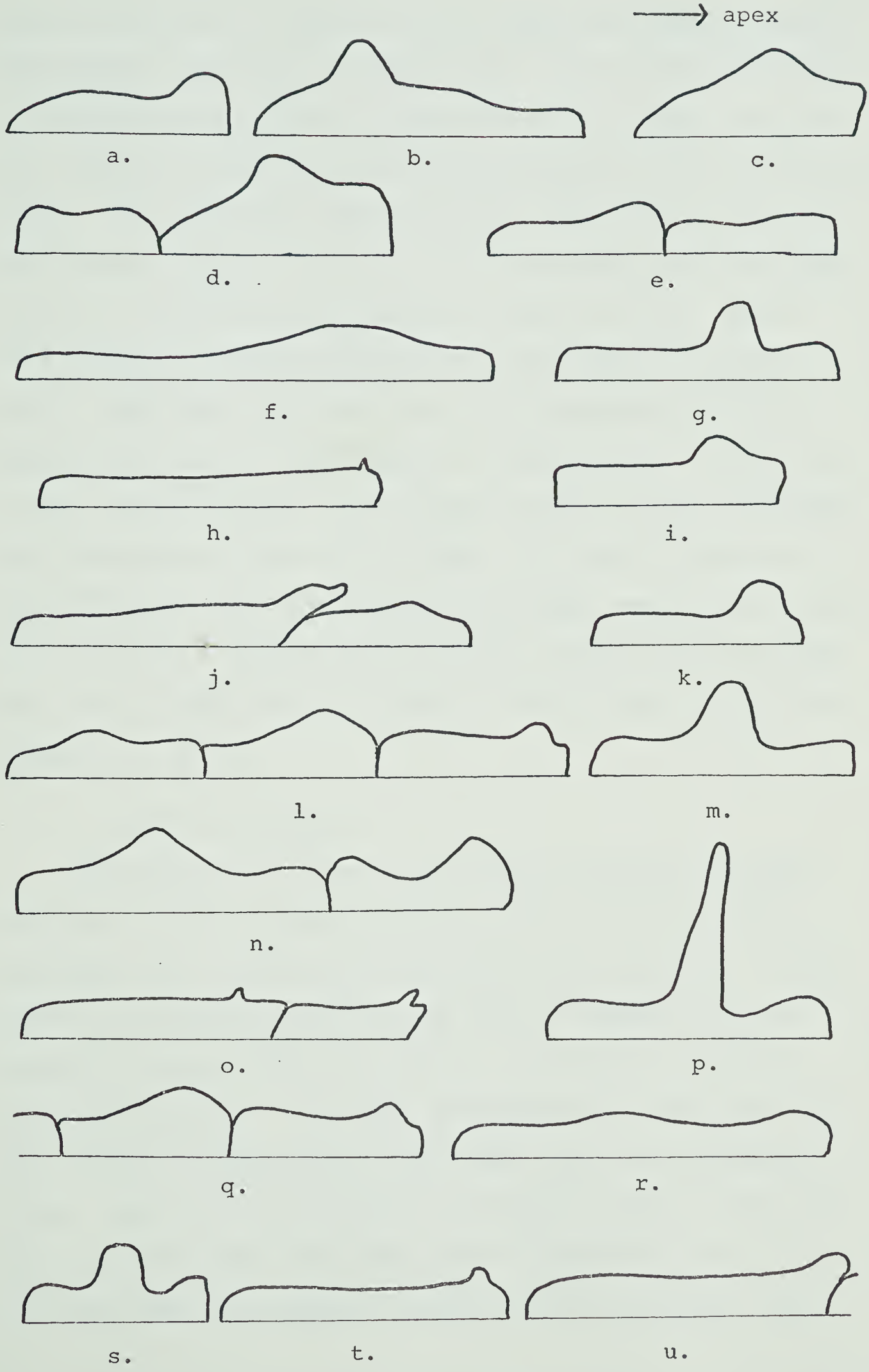
r. $10^{-6.5}$ M ammonium oxalate

s. $10^{-6.5}$ M ammonium oxalate

t. $10^{-7.5}$ M ammonium oxalate

u. $10^{-7.5}$ M ammonium oxalate

FIGURE 16



aqueous solution is found to be an important factor, maximum development occurring at a pH of 7.0. At this pH most roots are densely haired from the top to the tip. These are the most densely haired roots observed during the whole investigation. The root hairs themselves are long, narrow and very thin-walled (Figure 17, a, b, c). Decreasing or increasing the pH of the solution by means of borate buffers, even very slightly, greatly inhibits the development of root hairs. The fact that root hairs are developed only in a neutral solution of lipoxygenase is significant in that 7.0 is the optimum pH for the activity of this enzyme (Worthington Biochemical Corporation, 1968). At a pH of about 6.0 the epidermal cells are regularly arranged and hairless, and it is only occasionally that they are slightly swollen and give rise to very short papillae or short sharp projections (Figure 17, d, e).

4. Effect of Oxygen.

The amount of oxygen in the neutral aqueous solution is found to have a marked effect on the growth of onion seedling roots, and on the development of root hairs. Root growth is greatest in boiled tap water, somewhat less in slightly aerated boiled tap water, and least in aerated unboiled tap water. The best development of root hairs in this series of experiments also takes place in boiled tap water. Root hairs are densely developed on most roots and they are very short and fairly wide (Figure 18, a, b, c). On roots grown in slightly aerated boiled tap water, only

FIGURE 17

Drawings of epidermal cells of onion seedling roots showing the development of root hairs, papillae, and swellings occurring in solutions containing the enzyme lipoxygenase.

X 450

a. 1/2 mg lipoxygenase per liter at pH 7.0.

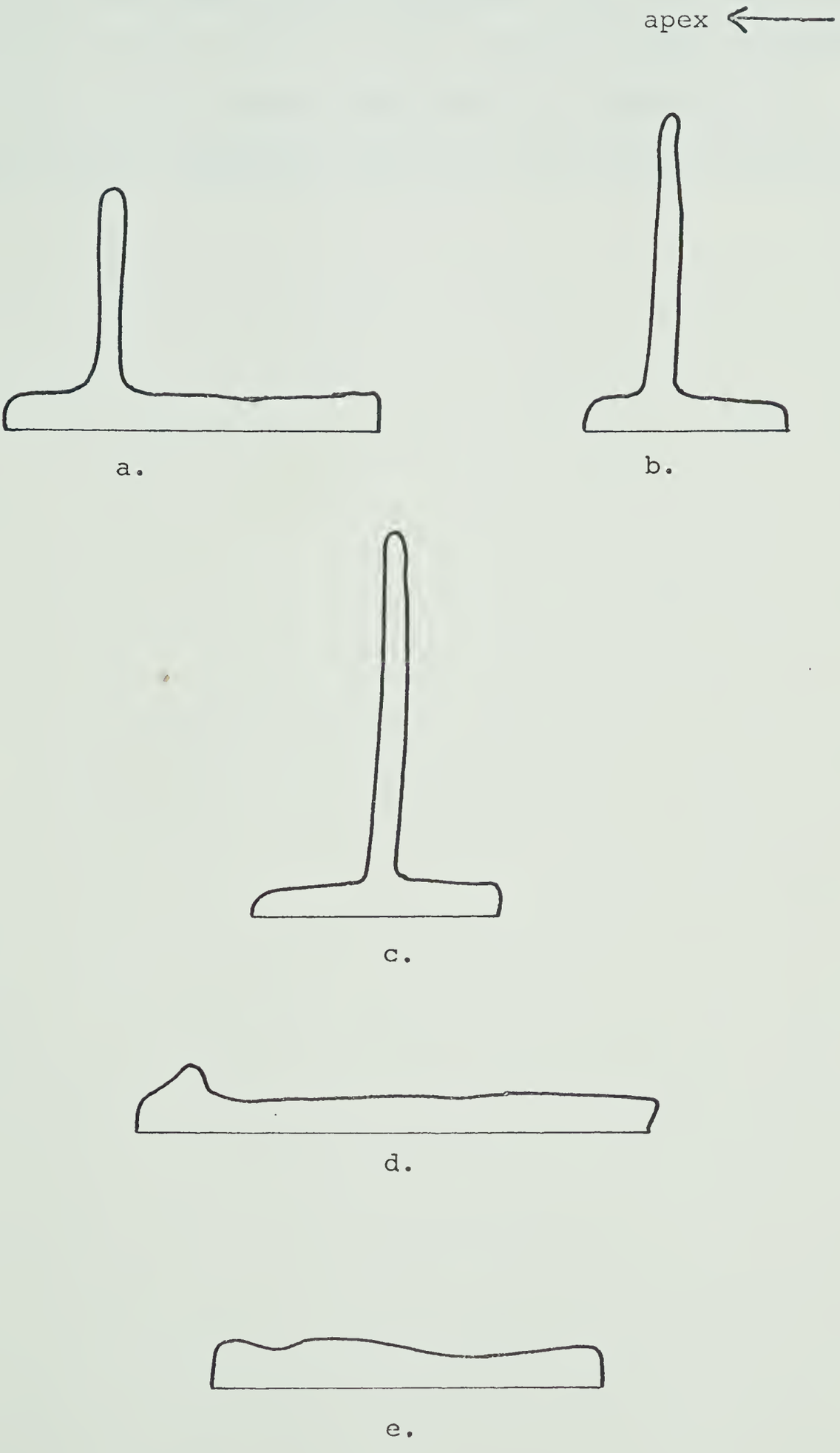
b. 1/2 mg lipoxygenase per liter at pH 7.0.

c. 1/2 mg lipoxygenase per liter at pH 7.0.

d. 1/2 mg lipoxygenase per liter at pH 6.0.

e. 1 mg lipoxygenase per liter at pH 6.0.

FIGURE 17



papillae are developed and the epidermal cells are occasionally swollen (Figure 18, d, e, f). On roots grown in aerated unboiled tap water, neither short hairs nor papillae are developed and the epidermal cells are not swollen (Figure 18, g).

FIGURE 18

Drawings of epidermal cells of onion seedling roots showing the development of root hairs, papillae and swellings occurring in tap water with various amounts of oxygen. X 450

a. Boiled tap water

b. Boiled tap water

c. Boiled tap water

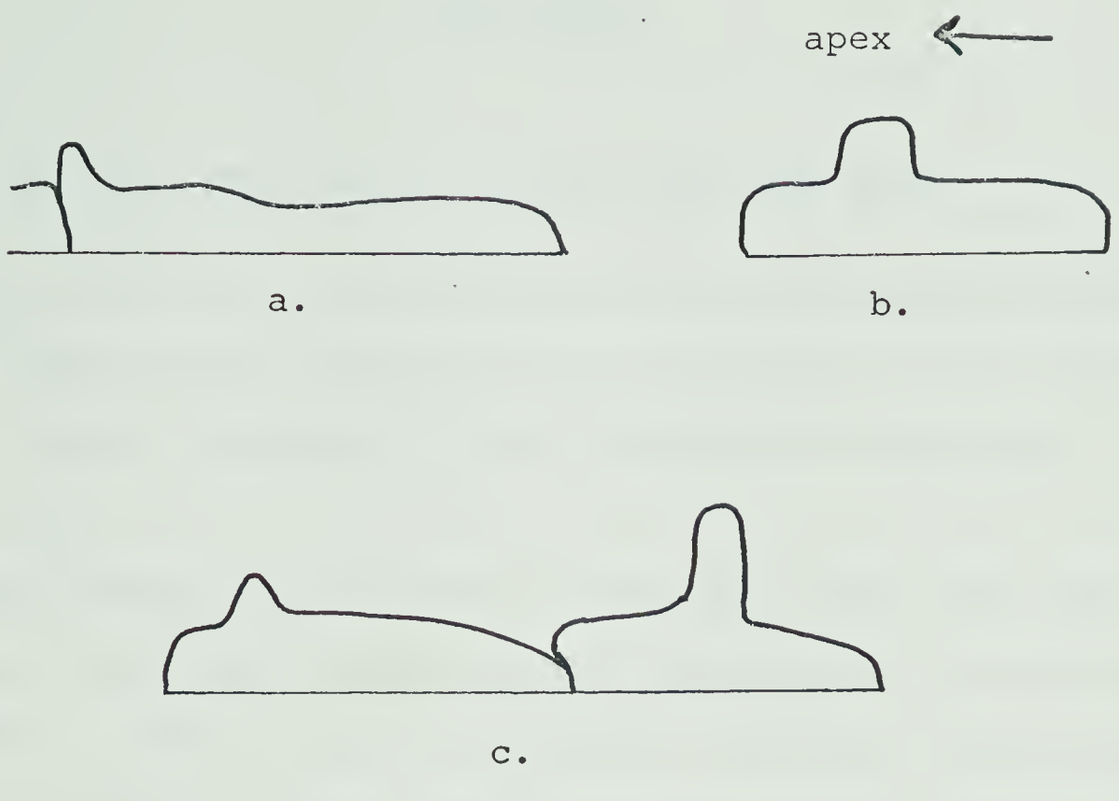
d. Slightly aerated boiled tap water

e. Slightly aerated boiled tap water

f. Slightly aerated boiled tap water

g. Aerated tap water

FIGURE 18



DISCUSSION

A. The Culture and Growth of Onion Seedling Roots

Any differences between the results obtained by Hoffman (1933) and Hayward (1938) and those obtained in this study can be readily explained by the difference in varieties of onion used.

The frequent occurrence of a swelling just above the root cap following transfer of the seedlings to the culture solution is not so readily explained. That the cells in the region in which swelling occurs are usually very short lends support to the idea that the apical cells, at the time of transfer to the solution, did not increase in length as much as normally but instead increased radially. Jensen and Kavaljian (1958) noted a rapid radial enlargement before elongation began. Extreme radial expansion would explain the occurrence of patches of epidermal cells being absent. Since the presence of a cuticle is indicated by the results of this study, the epidermal cells can be thought of as having a rigid coating, which is deposited very near the apical cells. If the root cells expand radially the diameter of the root also expands. This would result in a tension being exerted on the rigid cuticle which would then tear.

Since it has been shown that the cuticular material not only forms a layer over the epidermal cell walls but impregnates the epidermal wall as well, tearing of the cuticle results in the tearing of the epidermal cell wall,

and the exposure of the hypodermis.

A possible explanation for the hypodermal cells not being torn off during radial expansion is that they are suberized somewhat later than the epidermal cells. If the cell walls of the hypodermal layer were not heavily suberized at the time of the root's radial expansion then they could also stretch easily, and hence would not tear off.

Carlton (1943) observed swelling of onion roots treated with various growth-regulating substances. He attributed this increase in root diameter to cell enlargement. Of particular interest is his discovery of extreme hypertrophy of some of the hypodermal cells. Burström (1942) felt that rupturing of the epidermis of wheat roots was due to extensive enlargement of the hypodermal layer. In the present study, however, no extreme hypertrophy of the hypodermis was observed.

The actual causes of the decrease in elongation of the cells and the increase in radial growth remain unexplained. These causes would seem to be associated with some mechanism related to turgor pressure, because once the roots have had time to adjust to the culture solution the root resumes its normal diameter.

B. The Anatomy of the Seedling Root

This study confirms the conclusion drawn by Mer (1884), Rosene (1954), and Scott *et al.* (1958) that onion roots grown in water are hairless. The epidermis of the onion

root is undifferentiated and reinforces the pattern, already observed (Cormack, 1959b), that roots with an undifferentiated epidermis tend to be hairless. It was also found that contact with filter paper is necessary for the production of root hairs when the roots are grown in moist air. The reason for this phenomenon is unknown, but it was also observed by Rosene (1954).

The occurrence of a differentiated hypodermis confirms Guttenberg's (1943) observation. However, he maintained that the walls of the long hypodermal cells are suberized while the walls of the short hypodermal cells are not suberized. In this study it was found that the walls of both long and short hypodermal cells are heavily suberized.

Hayward and Long (1942) noted the formation of root hairs from the suberized hypodermis of *Citrus* roots. Although in onion seedling roots the differentiated hypodermis is frequently the outermost layer of cells due to the rupture of the epidermal layer, the hypodermal cells are always hairless.

C. Microchemical Studies

The microchemical studies are most revealing. All the histochemical tests, except the ruthenium red test for pectic compounds, are negative when performed on fresh roots, but the same tests are positive when performed on roots which have been stored in xylene. This indicates the presence of an ubiquitous, xylene soluble material which

prevents the microchemical reactions. The experiments with chromic acid indicate that this ubiquitous material is of a waxy, cutinous or suberinous nature.

The meristematic cells contain a great deal of fatty materials. The deposition of the waxy substances begins a very short distance above the meristem in very young tissues. Suberization of the hypodermis begins on the outer tangential walls. It then proceeds to the radial walls, and finally to the inner tangential walls. In the cortex those cells which are in the rows radiating outwards from the passage cells, are clearly suberized. One explanation for this is that because conditions are not suitable in the area of the passage cells for the oxidation of the fatty acids, these fatty acids are free to radiate out from the stele into the cortex where they are oxidized to form suberin. Van Fleet (1961) found that in the differentiation of the endodermis, fatty and phenolic substances are deposited in the endodermal cell walls where they are later oxidized to form suberin. In order for oxidation to occur, the cells must be at the optimum pH and the necessary enzymes must be activated. One of these enzymes is lipoxygenase. The acid condition at the protoxylem points does not favour suberization. Consequently the suberin precursors are free to diffuse outwards from the stele to the cortex where oxidation to suberin takes place. These fatty acids, or suberin, may correspond to the lipid component of the intercellular tubular matter observed by Sorokin (1958) in the intercellular spaces in

the vicinity of the vascular tissue of pea stems.

Scott (1950) and Scott and Lewis (1953) describe the presence of a "ubiquitous suberin pellicle" in the roots of several genera. In the present study of onion seedling roots the results of the experiments with cold chromic acid show that only parts, not all, of the cortex are definitely suberized.

Treatment with cold chromic acid reveals a thick rough cuticle over the epidermis. Treatment with IKI-H_2SO_4 reveals a granular material outside the outer epidermal cell wall. This material appears to have a multitude of minute projections and may correspond to the mucilage observed by means of the electron microscope by Scott *et al.* (1958). Or again it may be due to either the fat droplets or the pits in the cuticle which were noted at the same time by the same workers.

In onion seedling roots the entire epidermis, including the root hairs, is covered with a cuticle. However, the thickness of the cuticle is not uniform. It varies in thickness in different regions of the same root, and in different parts of the same cell. In general, the longer the epidermal cell the thinner the cuticle. This view is strengthened by the observation that following treatment of the epidermis with xylene the first cells to give a positive test for cellulose in their walls are the longest epidermal cells. This suggests that cutinization of the epidermal cell walls has an inhibiting effect on cell elongation.

That individual epidermal cells are not uniformly cuticularized is shown by the results of microchemical testing with zinc-chlor-iodine following extensive treatment with xylene. The highly variable degree of cutinization of the epidermal cell walls may partially explain the great range in the size and shape of the epidermal cells and the ability or failure to form root hairs observed in all the experiments conducted in the present study.

In agreement with the findings of Scott (1950), a cuticle is observed over the whole surface of the hair-forming epidermal cell, including the very tip of the growing hair. Once again the cuticle varies greatly in thickness. The presence of a cuticle over the entire root surface suggests that the view of the cuticle as an impervious layer may have to be reconsidered.

The only observable difference between roots grown in water and those grown in sand or between moist filter paper is in the amount of cuticle. Roots grown in water appear to have a somewhat thinner cuticle than those which have more contact with air. This is to be expected since the cuticle is presumably formed by the oxidation of fatty acids.

The cell walls of the epidermal cells and root hairs contain, in addition to cellulose and cutin, pectic compounds. The results of treatment with ruthenium red are somewhat obscured by the cutin, but this masking effect is not nearly as pronounced as it is in the other histochemical tests. Perhaps the reason for this is that the

pectic substances may not be totally beneath the cuticle. There may be pectic compounds within the cuticle itself or perhaps the mucilagenous layer may contain pectic substances. This view is supported by the observation by Norris (1971) of the presence of finger-like projections of what appears to be pectic compounds into the leaf cuticle of several species of plants. Scott *et al.* (1958) felt that the pectic layer was entirely beneath the cuticle, but the histochemical results of this study would suggest that this is not so.

The partial masking effect of the cuticle may explain why the older epidermal cells and root hairs stain less deeply with ruthenium red than do the younger epidermal cells nearer the apical end of the root. The older cell walls may have a thicker cuticle on them. Hence, the histochemical reaction on the pectic layer described by Scott *et al.* (1958) is not as pronounced as it would be, were the cuticle not as thick.

All the epidermal cell walls contain pectic compounds. Those cells which form hairs stain more deeply than those cells which do not. These lightly stained cells are on the whole longer than the haired cells. The root hairs themselves have pectic compounds along their entire length. The bases of the hairs stain more densely than do the tips.

The difference in the staining of the hair-forming and of the hairless cells may, in part, be due to a partial masking by cutin, since this difference is not as obvious following treatment of the roots with xylene.

In the hypodermal layer a marked difference was observed in the reaction of the cell walls of the long and the short cells. The short cells were much more deeply stained. This, too, may in part be due to the fact that these short cells have less suberin than the long cells. Guttenberg (1943) on the other hand maintained that the short cells have none. Part of the difference in staining must however be due to the fact that the short cells actually do contain more pectic compounds because following prolonged treatment of the roots in xylene there is still some difference in the degree of staining. The presence of calcium pectate in the walls of the short hypodermal cells may explain why these cells do not elongate to the same extent as do the long hypodermal cells.

D. Effects of Various Culture Solutions on Root Growth and Root Hair Development

1. Effect of Calcium and pH.

There is general agreement that calcium stiffens or hardens the walls of elongating root epidermal cells (Cormack, 1935-1962; Farr, 1928, 1929). There is also considerable evidence that the amount of calcium and the pH modifies the rate at which the change from pectic acid to calcium pectate takes place. In the roots of *Brassica* spp. which produce an abundance of root hairs in tap water, acid solutions were found to inhibit the formation of root hairs by slowing down the change to calcium pectate; slightly alkaline solutions were found to stimulate the formation of

root hairs by speeding up the change from pectic acid to calcium pectate while strongly alkaline solutions caused rupturing of the epidermal cells by speeding up the change to calcium pectate too rapidly (Cormack, 1935, 1962; Farr, 1928, 1929; Lemay, 1965).

The observations of the effects of calcium and pH on the roots of onion seedlings are at variance with those obtained with *Brassica* roots. Concentrations of calcium above that found in tap water inhibit root hair formation. Alkaline solutions inhibit the formation of root hairs whereas acid solutions stimulate the formation of root hairs. Differences in the reaction of *Brassica* root epidermal cells and onion root epidermal cells to calcium concentration and pH must be attributed to differences in the chemical nature of the root epidermal cell walls of these two plants. The epidermal cell walls and the walls of the hairs of *Brassica* roots consist of cellulose and calcium pectate while the epidermal cell walls and the walls of the root hairs of onion seedling roots consist of cellulose, pectic compounds and cutin. When the roots of onion seedlings are induced to form root hairs, a thin cuticle is observed over the whole surface of the hair, including the very tip. This observation suggests that the epidermal cell walls on onion seedling roots, strengthened by means of a cuticle, are too stiff or too firm to respond to conditions which lead normally to the development of root hairs. Evidence obtained by Van Fleet (1961) in his study of the root endodermis showed that fatty substances are not

oxidized to form suberin in an acid medium with a positive redox potential. The observation that onion seedling roots are stimulated to produce hairs in an acid medium is in keeping with these findings of Van Fleet (1961).

There is little doubt that the stiffening of the epidermal cell walls of onion seedling roots through cutinization complicates the situation. The reaction of the epidermal cells to the various culture solutions indicates that the formation of root hairs by this plant is not only dependent upon conditions that affect the pectic substances but upon those that affect the fatty substances as well.

2. Effect of Calcium Deficiency.

The experiments with the calcium chelating agents, Na_2EDTA and ammonium oxalate, throw further light on the chemical nature of the cell wall as it affects the development of root hairs on roots of onion seedlings. The general effect of Na_2EDTA is to reduce root hair formation and to cause marked swelling and separation of the epidermal cells (Cormack, 1959a, 1959b). Both swelling and separation is attributed to the chelation of the calcium ions before they can combine to form calcium pectate in the elongating cell walls.

In the present study, the formation of papillae when roots of onion seedlings are grown in dilute solutions of Na_2EDTA or ammonium oxalate indicates that here too the effect of chelating agents is to render the epidermal cell walls more pliable. However, microchemical tests on the epidermis

of onion seedling roots grown in solutions of Na_2EDTA show that a cuticle is always present and that it is thicker on roots grown in alkaline solutions than on roots grown in acid solutions. In strongly alkaline solutions, papillae are absent and the epidermis is often ruptured. The rupturing of the epidermal cell walls under these conditions, where Na_2EDTA is present, cannot be attributed to the stiffening of the wall by calcium pectate, but to the existence of a very thick cuticle which develops in an alkaline medium. Van Fleet (1961) concluded that an alkaline medium facilitates the oxidation of fatty acids to suberin. The results obtained with onion seedling roots in alkaline solutions are in keeping with this viewpoint.

The whole series of experiments with solutions of chelating reagents strengthens the veiw that the epidermal cell walls of onion seedling roots are too stiff under most conditions to push out to form hairs, and that this stiffness is due both to the oxidation of fatty acids to form a cuticle, and to the calcification of the pectic compounds to form calcium pectate.

3. Effect of Lipoxygenase.

There is considerable evidence of the occurrence of fatty materials in both epidermal and root hair cell walls (Cormack, 1937; Dale, 1951; Dawes and Bowler, 1959; Scott, 1950; Scott *et al.*, 1958). In the present study the experiments with aqueous solutions of lipoxygenase furnish further evidence of the occurrence of fatty materials in

the epidermal and root hair cell walls, and strengthen the view that they have a direct bearing on the development of root hairs in the roots of onion seedlings. Roots grown in this solution were the most densely haired roots observed during this investigation. Most roots grown in solutions of this enzyme at a pH of 7.0 are densely haired from top to tip.

Microchemical tests reveal the presence of a thin cuticle over the whole surface of the epidermis and over the whole surface of the hairs, including their very tips. The cuticle is thicker on hairs which are approaching maturity than on actively growing hairs. At present it is not clear how this enzyme influences the metabolism of fatty substances in the walls of living cells. The fact that the reaction takes place within a narrow range of pH suggests that lipoxygenase here is interfering in some way or other with the oxidation of fatty acids to form cutin. Whatever the cause, the end result appears to be the softening of the outer wall which in moist air, or in water, is generally too stiff to form a hair.

4. Effect of Oxygen.

The view as to the formation of a cuticle as expressed by Lee and Priestley (1924) seems most acceptable in the present state of knowledge. They believe that it is developed by the migration of fatty acids from the protoplasts of both internal and external tissues to the surface of the epidermis where these fatty acids undergo condensation

and oxidation. Considerable evidence in support of this conception of cuticle development was obtained from the results of experiments on the development of root hairs by the water plant, *Elodea canadensis* (Cormack, 1937). The roots of this plant are completely hairless in water in the light but produce an abundance of hairs in soil or water in the dark. Roots grown in water and in light are green and are covered with a cuticle. Roots grown in darkness, whether in water or in soil are colourless and have no cuticle. The hypothesis expressed is that the tough cuticle prevents root hair formation and for the production of this cuticle under water the oxygen released by photosynthesis in the cells of the root is necessary.

Although the experiments with oxygen in the present study were of a cursory nature, the results show that the amount of oxygen in the aqueous solution has a marked effect on the development of hairs by the roots of onion seedlings. To summarize, root hairs are produced in boiled tap water, papillae only are produced in slightly aerated tap water and neither hairs or papillae are produced in well aerated tap water. That oxygen acts through its role in the development of a cuticle seems highly probable in the light of the results of microchemical tests. When the cuticle is thin, hairs are produced, when the cuticle is thick, the roots are hairless. These observations are in agreement with the viewpoint as expressed by Cormack (1937), "that the cuticle is a tough non-plastic substance and once it has been formed

its presence would be sufficient to prevent the lateral extension of the cell wall in the form of a hair."

The observation of other workers reported by Belford and Preston (1961) that actively growing root hairs burst at the tip when transferred to solutions of low oxygen content may be explained by the role oxygen plays in the formation of a cuticle on the cell wall of the root hair. Perhaps, when oxygen is lacking, a cuticle cannot be formed on the tip of the root hair. In this case the growing cell wall at the tip of the root hair is so weak that it bursts.

In conclusion, the results of the present study on the development of root hairs by the roots of onion seedlings confirm the observations of Dawes and Bowler (1959) and Scott *et al.* (1958) that the walls of the root hairs, and the walls of the epidermal cells which give rise to them consist of cellulose, pectins, and a cuticle.

Electron microscopic studies of the tip of growing root hairs (Belford and Preston, 1961; Dawes and Bowler, 1959; O'Kelley and Carr, 1954) reveal cellulose microfibrils right to the tip of the hair where growth is occurring. There is no questioning the strengthening properties of cellulose but there is increasing evidence that differences in cell wall plasticity are more closely associated with differences in the chemical and physical nature of the non-cellulosic substances in the cell wall. In this connection, Wardrop (1962) regards "the non-cellulosic matrix as the most important structural component determining the growth of

primary cell walls."

The understanding of changes in cell wall plasticity as associated with the development of root hairs by onion seedling roots is complicated by the presence of both fatty and pectic substances in the epidermal cell walls. On one hand, the swelling of epidermal cells in solutions deficient in calcium and the inhibition of root hairs in calcium solutions indicate that the pectic substances play some part in regulating cell wall plasticity. On the other hand, the development of root hairs under conditions that hinder the oxidation of fatty acids to cutin and the inhibition of root hairs under conditions that stimulate fatty acid oxidation, indicate that the fatty substances also play a part.

As stated earlier, the exact role of the enzyme lipoxygenase in affecting the most dense development of root hairs observed in this study is not clearly understood. That it is connected in some way with the development of a thinner, and much weaker cuticle seems highly probable in the light of the results of the microchemical tests. There is little doubt that both fatty and pectic substances are associated with differences in cell wall plasticity in onion seedling roots. However, the presence of a cuticle in the walls of hairless epidermal cells, in the walls of the hair-forming cells and in the walls of the hairs themselves, emphasizes the important role played by fatty substances. With reference to the role of these substances in the differentiation of tissues, Sifton (1957) concludes that, "such a

layer...if composed of polymerized fats might well, through its rigidity, be a factor in determining the final size and shape of cells and of intercellular spaces."

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